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Contents

Studies on the Nerve Endings in the Extrinsic Eye Muscles of the rabbit ..... *Masatoshi Fukuda*

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Studies on the Action of the Extraocular Muscles in Monocular Movements by Means of Quantification of Integrated EMG ..... *Hirofumi Momosse*

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5

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Contents

Studies on the Nerve Endings in the Extrinsic Eye Muscles of the Rabbit ····· *Masatoshi Fukuda*··· 93

A Histological Study on the Distribution of Nerves in the Human Lacrimal Gland ····· *Yoshiko Kato*···103

Studies on the Action of the Extraocular Muscles in Monocular Movements by Means of Quantification of Integrated EMG ····· *Hirofumi Momosse*···108

A Clinical Study on Behçet's Syndrome ····· *Tadashi Kato*···122

Metabolism in Inner and Outer Layers of the Retina ····· *Yoshi Kurachi, Kiyoshi Saito & Nobuhiko Kozawa*···127

Experimental Studies on Twilight Vision and Road Safety ····· *Atsuko Nomura*···134

Experimental Studies on Chronic Retrobulbar Neuritis ····· *Jo Imachi, Issaku Kajikawa, Shuichi Matsumoto & Hisao Ito*···143

Studies on the Central Retinal Arterial Pressure ····· *Masahiko Sasamoto*···154

Impedance Test of the Corneal Tissue ····· *Kiyoshi Hara, Shoji Taoka & Sachio Kida*···160

The Arthus Phenomenon in the Cornea ····· *Shin-ichi Shikano*···166

Incorporation of Inorganic S^{35}-Sulfate into Sulfated Mucopolysaccharides in the Rabbit Cornea ····· *Makoto Suda*···172

STUDIES ON THE NERVE ENDINGS IN THE EXTRINSIC EYE MUSCLES OF THE RABBIT

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In relation to the control of ocular movement, it is an interesting and important problem whether the muscle proprioceptors are present or not in the extrinsic eye muscles.

The purpose of this work is to investigate histologically the nerve endings in the rabbit's extraocular muscles, to trace the origin of these nerve endings and fibers, and to assist in the preceding work of the same author which described the ganglion cells in the roots of the 3rd, 4th and the 6th cranial nerves.

Surveying the literature upon the nerve endings in the muscles, various kinds of nerve endings have been described by several authors in the extraocular muscles of different animals. But their opinions have not coincided with each other upon the sensory nerve endings which Sherrington hoped to find and to confirm his hypothesis on the spatial perception. Spiral nerve endings which are similar to the muscle spindles in ordinary skeletal muscles were found in the eye muscles of man, higher apes, and the artiodactyles, but in the other mammalian eye muscles they have not been found. In the rabbits eye muscles these spiral nerve endings are absent, but the "terminaison en grappe" which was described first by Retzuis is present and it has been suggested that this ending resembles morphologically sensory endings as well as the spiral nerve endings in the preceding literature.

Material and Method

Materials used comprise all the extrinsic eye muscles of the rabbit, i.e. the superior, inferior, lateral and medial rectus muscles, the superior and inferior oblique muscles and the retractor.

In the first place, normal extraocular muscles were taken from 4 adult unoperated rabbits as well as from the unoperated side of the experimented rabbits. In the second place, unilateral degeneration experiments were carried out on 25 rabbits and with the lapse of from 2 to 34 days these materials were removed in order to examine the degeneration of these endings.

1) When the oculomotor nerve trunk was cut at the exit from the brain in 15 rabbits, the time allowed for degeneration was 2 to 28 days.

2) The sympathectomy was carried out on 5 rabbits and with the lapse of from 11 to 34 days these eye muscles were removed.

3) After the intracranial severance of first division of trigeminal nerve just distal to Gasserian ganglion, 4 rabbits were kept alive for 5, 7, 10 and 14 days respectively.

4) In the case of double operation, the section of the nervus oculomotorius at the base of the brain was carried out on a rabbit which had been kept alive for 38 days after

the sympathectomy and with lapse of 4 days these materials are removed.

The technique of the above mentioned operations have been described by the same author in his preceding report. One half of these materials were fixed about one month in a solution of 10 per cent neutral formalion, embedded in paraffin, cut in serial sections and stained by the silber impregnation (of Bielschowsky) method.

The other half of them were studied with methylene-blue vital staining method. In this method, muscles removed freshly were placed as quickly as possible in the solution of 1 : 5000 methylene-blue in Linger solution, after one hour placed in the fixative (5 per cent ammonium molybdate), in which they remained for 24 hours. And these muscles were washed in distilled water, pressed between two pieces of objectglass, and the permanent preparations were made by running these muscles through the alcohol and xylol, and xylol, and mounting them in balsam.

Furthermore, the superior and medial cervical ganglion and its chains were removed in two rabbits, and with the lapse of 14 and 20 days the orbital muscles and the palpebral smooth muscles (Müller's muscles) were studied with the methylene-blue vital staining.

In this case a solution of 1 : 10000 methylene-blue in Linger solution was injected through the cannula which was inserted into the carotid artery of these rabbits and two liters of these solution which was heated to body temperature were flowed. After the blood had been washed out, all the contents of the orbit with the periosteum of the orbit were removed as quickly as possible. The preparation stood thus exposed to the air for one hour, and then these muscles were detached. The rest of this method conforms to the preceding method.

Observations

On the appearance of nerve endings in the normal extra-ocular muscles. In the rabbits normal extrinsic eye muscles, five morphologically distinct types of nerve endings, excepting those found on blood vessels, were encountered, they are: 1) Nerve endings originating from thick medullated nerve fibers. They apparently represent the motor end plates of Kühne etc. and seem to be hypolemmal. 2) Nerve endings originating from thin medullated nerve fibers. They correspond with the "terminaison en grappe" or the "atypical motor ending" of Retzuis and are undoubtedly epilemmal. Each thin nerve fiber runs along the muscle fibers solitarily giving off fine branches which are supplied to these termini, and the end of this nerve seems to run into the muscle fiber. 3) Complicated fine nerve endings in whose formation two non-medullated or fine medullated nerve fibers take part. The fine nerve fiber runs into the eye muscles together with the thick medullated fibers which were supplied to the motor end plates and separated from these nerve fibers before their end plates. The branches of this fine nerve fiber form this nerve ending which seems to be hypolemmal. 4) Simple fine nerve endings originating from fine nerve fibers probably of autonomic nature. These fine fibers run about lengthwise and crosswise on the muscle fibers, giving off finer branches which seem to be beady in appearance, and often connect with the

nerve fibers innervating blood vessels. 5) Accessory terminations which are rarer than any other endings. They belong to fine fibers which originate from inside the motor end plate and these fibers extend to other muscle fibers beyond the area of the original end plate.

The muscle spindle is absent. The motor end plates occur commonly in the proximal third, and the other nerve endings are found mainly in the middle as well as in the distal third of each muscle.

On the appearance of nerve terminations after the unilateral degeneration experiment. When the oculomotor nerve trunk was cut at the exit from the brain, the nerve endings and their supplying nerve fibers degenerated completely, excepting those thin nerve fibers which were found on blood vessels. Some branches of those intact fibers were traced to endings in the ocular muscle fibers. In case when the superior cervical ganglion and its chains were removed, nerve terminals on the muscle fibers were not affected, and only those which innervated small blood vessels were lost. All muscles were denervated completely by the combination of the oculomotor section and sympathectomy. By the intracranial severance of first division of the trigeminal nerve just distal to Gasserian ganglion, all nerve endings and fibers exhibited no departure from the normal appearance. And in these experimented animals the inferior oblique branches of the oculomotor nerve were studied by the staining and fixation of the osmiumic acid in which these fresh materials were placed for half an hour. In the case of oculomotor section all nerve fibers degenerated completely; on the contrary, they did not degenerate in other cases (sympathectomy and trigeminal section).

On the appearance of nerve terminations within the extraocular smooth muscles (the orbital and the palpebral smooth muscles). The orbital and the palpebral smooth muscles were studied with vital staining method, and fine nerve fibers and their terminations which were probably of the autonomic nature were found in these muscles.

These fine nerve fibers run about lengthwise and crosswise on the muscle fibers as well as the nerve fibers which were supplied to the simple fine nerve endings in the extrinsic eye muscles.

But in case when the superior and medial cervical ganglion and its chains were removed, these smooth muscles were denervated completely on the operated side.

Discussion

It is shown that five morphologically distinct types of nerve endings found in the rabbit's extraocular muscles are supplied with nerve fibers either from the ocular motor nerve trunk or from the cervical sympathetic ganglion.

In the rabbit's eye muscles muscle spindle is not found, but the terminaison en grappe of Retzius seems to correspond with sensory nerve endings described by Huber, Hines etc.

These nerve endings originating from thin medullated nerve fibers are looked upon as sensory endings from the following reasons:—

1) These medullated nerve fibers are different from those fibers supplied to the motor nerve endings.

2) These endings seem to be epilemmal and resemble in structure the terminal branches of medullated nerves terminating in known receptors of skeletal muscles.

3) These endings are present near the tendinous insertion of the eye muscle fibers.

4) There are no other nerve endings which resemble sensory endings in the extrinsic eye muscles of the rabbit.

Kure, Shiba & Okinaka (1931) described in the eye muscles of the dogs four types of nerve endings which were supplied with nerve fibers from the roots of pyramidal (motor), extrapyramidal, sympathetic and parasympathetic nerve. Sensory nerve endings were however not reported. In the literature upon the nerve endings in the eye muscles, extrapyramidal nerve ending has not been reported by previous authors excepting Kure etc. In the present work five morphologically distinct types of nerve endings are found, and the extrapyramidal endings have not been reported by previous authors.

The simple fine nerve endings resemble in structure the sympathetic nerve endings. The complicated fine nerve endings and the accessory terminations may be either of the extrapyramidal or of the parasympathetic nature. The nerve endings originating from thin medullated nerve fibers resemble in position the tendon receptors rather than the muscle spindles.

Next, the neurones supplying these endings were traced by the unilateral degeneration experiments.

According to these experiments the innervation of muscle fibers within the rabbit's extra-ocular muscles originates from both the 3rd cranial nerve and the cervical sympathetic and these muscles are not innervated by the trigeminal nerve.

The cervical sympathetic nerve innervate the blood vessels, the extrinsic eye muscle fibers and the extra-ocular smooth muscles i.e. the orbital muscles and the palpebral smooth muscles.

The search to find the central path of the first neuron from an eye muscle receptor did not prove to be easy in this work, though it may be concluded from the present and the previous reports which dealt the ganglion cells in the roots of the 3rd, 4th and the 6th cranial nerves, that the cells of origin of afferent fibers supplying the extrinsic eye muscles are not located peripherally but inside the brain stem. The finding of the degenerations experiments which were studied on the inferior oblique branches point out that all medullated nerve fibers supplying the ocular muscles run through the roots of the ocular motor nerve to their inlet of each muscles. Neurons of the ganglion cells which exist in the root of ocular motor nerve may be not contained in these ocular motor nerve trunks or not medullated fibers. Sympathetic nerve fibers penetrate the extrinsic eye muscles along its blood supply.

Summary

Studied with methylene-blue vital staining and silver impregnation method, five mor-

phologically distinct types of nerve endings, other than those found on blood vessels, were identified in the extrinsic eye muscles of the rabbit. They are; nerve endings originating from thick medullated nerve fibers, nerve endings originating from thin medullated nerve fibers, complicated fine nerve endings, simple fine nerve ending, and accessory termination. The nerve endings originating from thin medullated nerve fibers correspond with the "terminaison en grappe" of other writers and are looked upon as sensory endings. And the other nerve endings are probably of the pyramidal (motor), extrapyramidal, sympathetic and parasympathetic natures.

When the oculomotor nerve is cut at the base of the brain, these nerve endings and their supplying nerve trunks degenerate, excepting the simple fine nerve ending and the thin nerve fibers found on blood vessels. The combination of the oculomotor section and sympathectomy completely denervates the muscle, and by the intracranial severance of ophthalmic nerve just distal to Gasserian ganglion, all nerve endings and fibers exhibited no departure from the normal appearance.

Therefore the nerve endings and fibers within the rabbit's extrinsic eye muscles originate from both the ocular motor nerves and the cervical sympathetic, and the cells of origin of the afferent fibers supplying these muscles are located not peripherally but inside the brain stem.

(Published in Acta Ophth. Jap. 60:318, 1956, 61:51, 1957 in Japanese.)

Studies on the Nerve Endings

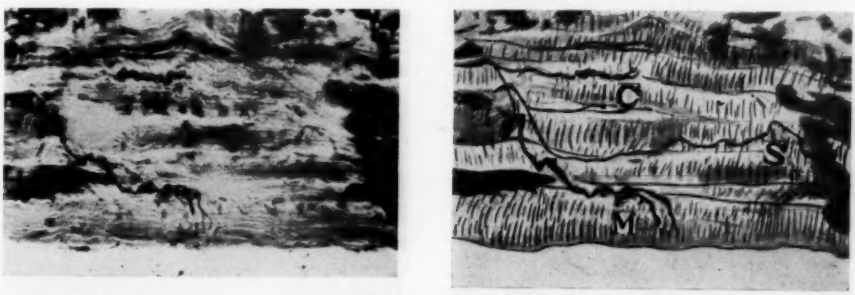


Fig. 1. Some of the nerve endings and nerve fibers in the normal rabbit's extrinsic eye muscle. (Bielschowsky's silver method.)
The right figure is a sketch of the left photograph. Both show a nerve ending originating from thick medullated nerve fiber (M.), the nerve endings originating from thin medullated nerve fiber (S.), and the complicated fine nerve ending (C.).

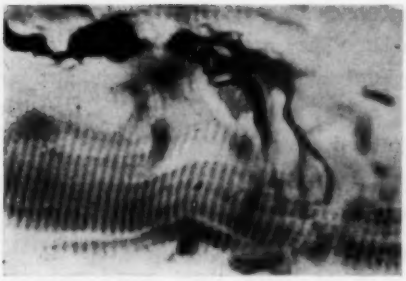


Fig. 2. A nerve ending originating from thick medullated nerve fiber upon a muscle fiber. (Bielschowsky's method.)

Fig. 3. The same ending as in figure 2 (↑). Methylene-blue vital staining method.)

Fig. 4. Nerve endings originating from thin medullated nerve fiber. A thinly medullated fiber of the initial medullated nerve fiber (↑), innervates a muscle fiber with the terminaison en grappe, descends diagonally along the muscle fiber and end on the right part of the same muscle fiber. (Bielsdhowsky's method.)

Fig. 5. Thin medullated nerve fiber and a terminaison en grappe. (Methylene-blue method.)

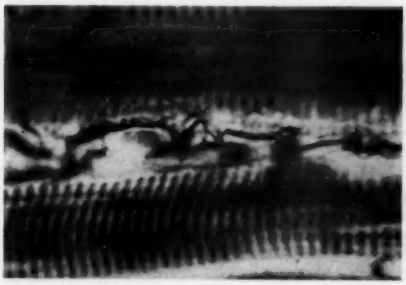


Fig. 6. The proximal part of the thin medullated nerve fiber with terminaison en grappe. Note the myelin sheath and the side branches at nodes of Ranvier. (Bielschowsky's method.)

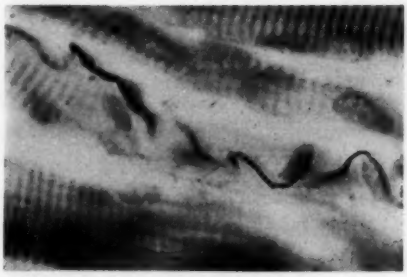


Fig. 7. The same part of the thin medullated nerve fiber as in figure 6, giving off side branches to the muscle fibers.
(Bielschowsky's method.)

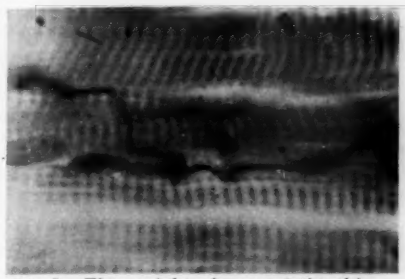


Fig. 8. The peripheral part of the thin medullated nerve fiber, giving off a nerve branch and a terminaison en grappe. (Bielschowsky's method.)

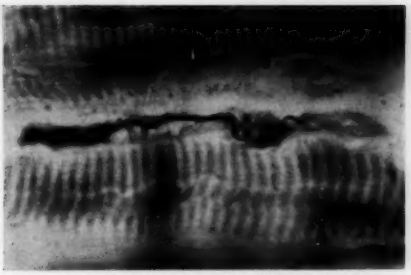
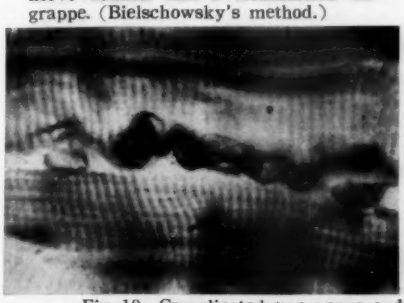


Fig. 9. The end part of the thin medullated nerve fiber on the muscle fiber, ended in a terminaison en grappe on the muscle fiber. (Bielschowsky's method.)



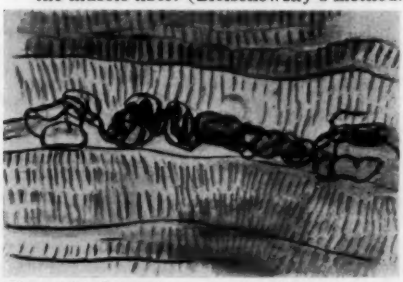


Fig. 10. Complicated fine nerve ending. The right figurs is an explanatory sketch of the left photograph.

Fig. 11. Accessory termination (A) on a muscle fiber, connecting with an upper motor end plate (M) on other muscle fiber. The right figure is its explanatory sketch. (Bielschowsky's method.)

Fig. 12. Fine nerve fibers (↓) supplying the simple fine nerve ending, running about lengthwise and crosswise on the muscle fibers. (Bielschowsky's method.)

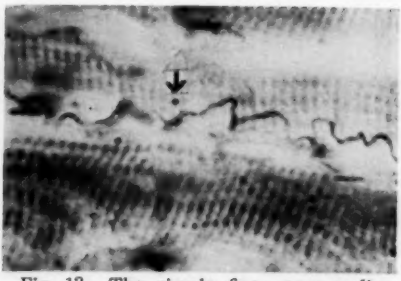


Fig. 13. The simple fine nerve ending (↓) and fine nerve fibers. (Bielschowsky's method.)

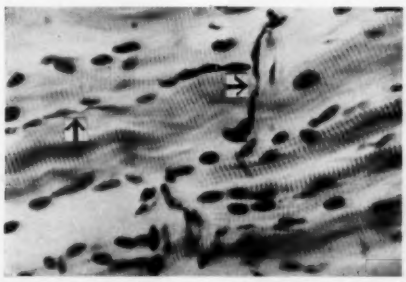


Fig. 14. The fine nerve fibers (↓) found on the capillary blood vessels between the muscle fibers. (Bielschowsky's method.)

Fig. 15. The degenerated nerve fibers and endings in a extrinsic eye muscle. Taken from the left superior rectus muscle, 3 days after cutting the left oculomotor nerve. The thick medullated nerve fibers (1), the thin medullated nerve fibers (2), and a nerve ending originating from thick medullated nerve fibers (M) are all degenerated. (Bielschowsky's method.)

Fig. 16. The degenerated nerve fiber and endings in an eye muscle. Taken from the left medial rectus muscle, 3 days after cutting the left oculomotor nerve, and showing 1, 2, M, the nerve endings originating from thin medullated nerve fiber (S) and accessory termination (A). (Bielschowsky's method.)

Fig. 17. The degenerated appearance of thick medullated nerve fiber (1) and its nerve ending (M). Taken from the left inferior oblique muscle, 11 days after cutting the left oculomotor nerve. (Methylene-blue method.)

Fig. 18. Fine nerve fibers found on the blood vessels, running along the arteriole between muscle fibers. Taken from the left superior rectus muscle, 11 days after cutting the left oculomotor nerve showing no departure from the normal appearance. (Methylene-blue Method.)

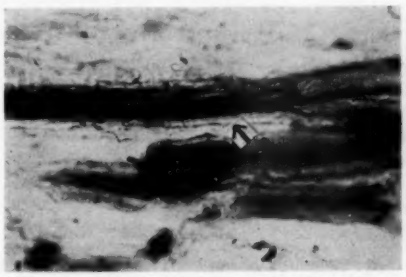


Fig. 19. Fine nerve fibers (↓) running around the muscle fibers. Taken from the left medial rectus muscle 28 days after cutting the left oculomotor nerve. (Bielschowsky's method.)

Fig. 20. Transverse section of a inferior oblique branch of the oculomotor nerve, taken from the left eye 15 days after cutting the left oculomotor nerve. All of the medullated nerve fibers are degenerated. (Osmiumic acid method.)

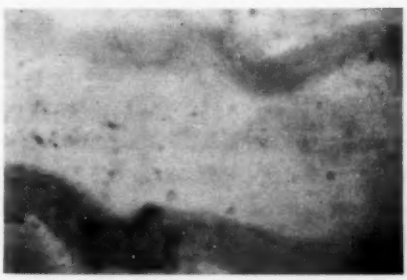


Fig. 21. Rabbit's palpebral smooth muscles (Müller's muscles) on the left. (Left sympathectomy, 20 days.) This smooth muscles are denervated, excepting the nerve endings which were found on superior palpebralis muscle. (Methylene-blue method.)

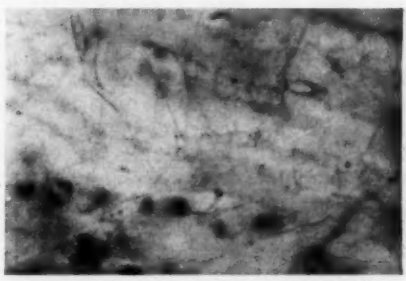


Fig. 22. The nerve plexus and ending within the right palpebral smooth muscle of the rabbit, the same animal as in figure 21. (Methylene-blue method.)

Fig. 23. Rabbit's orbital muscles on the left (left sympathectomy, 20 days). These smooth muscles are denervated completely, but a few number of the nerve fibers and endings on the conjunctiva are found in the same photograph. (Methylene-blue method.)

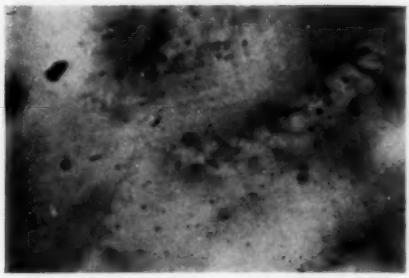


Fig. 24. Nerve plexus and endings within the right orbital muscle of the rabbit, the same animal as in figure 23. (Methylene-blue method.)

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A HISTOLOGICAL STUDY ON THE DISTRIBUTION OF NERVES IN THE HUMAN LACRIMAL GLAND

Yoshiko KATO

Department of Ophthalmology, School of Medicine, University of Tokyo

By means of silver impregnation methods nerve distributions and terminations in the human lacrimal glands were studied. Among various staining methods used, Seto's and Suzuki's modifications of Bielschowsky silver method were found to give satisfactory results for clarifying the relation between the glandular cells and the nerve terminations. Materials fixed in osmic acid and embedded in paraffin were examined for reference.

Results and Considerations

Nerve bundle. The nerve bundles distributing in the glandular tissues are composed of many non-medullated thin and a few medullated thick fibers. Most of the bundles wind along blood vessels, in particular following arteries. A few of them run independently through the interstitial connective tissue. At the ramifications of the bundles of various thicknesses, there commonly occurs a regular fiber exchange between the branches.

Reticular non-free nerve termination. The small nerve bundles or plexuses composed of thin non-medullated fibers divide further into finer and finer sub-branches, showing reticular arrangements of fibers around the effector elements, and finally pass gradually into the terminal reticulum composed mainly of extremely delicate varicose neurofibrillae. The terminal reticulum in which Schwann's or interstitial nuclei are scattered forms flattened bands and surrounds not only the basal surfaces of the acini and excretory ducts but also the surfaces of fat cells scattered in the glandular parenchyma. Furthermore, these nervous bands are apt to connect with the same structures surrounding the intraglandular blood vessels. Nevertheless, they neither enter the epithelium nor are connected directly with the protoplasm of the effector cells. Ramifications and mutual anastomoses occur repeatedly in the course of their distribution, and a non-free netlike terminal system of nervous nature is formed through the gland as shown in Fig. 1. fig. 2 shows the nerve plexus composed of non-medullated fibers and the terminal reticulum concerning the wall of the excretory duct.

In fact, the nature of the nerve fibers cannot be positively determined by the present method, but these nervous terminal reticula are similar rather to what Stöhr and Reiser recognized as "vegetatives Terminal-reticulum" than to what Boeke designated as "sympathische Grundplexus". Accordingly, it would be reasonable to regard this structure as representing the common endformation of the vegetative postganglionic components in the lacrimal gland.

Medullated nerve fiber ending (Non-relicular free nerve termination). After separating from the nerve bundles, medullated thick fibers, which have a smooth surface and are variant in thickness, approach their territory taking an irregular winding course. With a

Fig. 1.

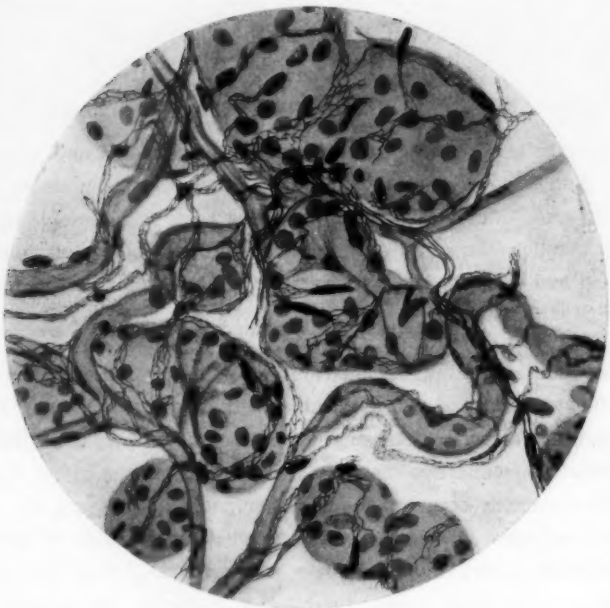
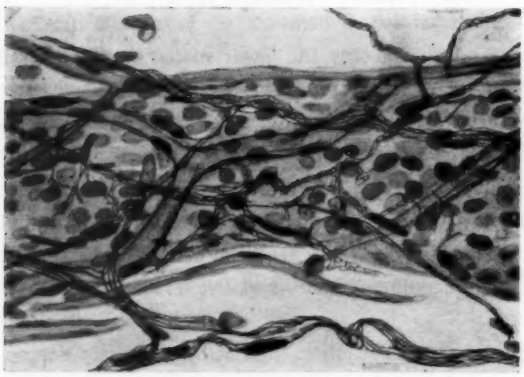


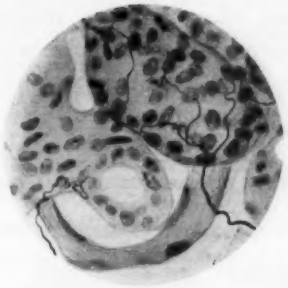
Fig. 2.



few exceptions, the terminal mode of these fibers is, as classified by Seto, divided into the simplest unbranched and the simple branched termination composed of a few branches. The terminal fibers (including their branches) are characteristic of non-reticular distinct free and sharp ending, whether they are subepithelial or intraepithelial. It is supposed that the medullated thick fiber is sensory in function.

1. Subepithelial termination. The terminal fibers apparently end on the basal surface of the secreting portion as shown in fig. 3 or of the excretory duct. It is, however, rather difficult to ascertain whether the endpoint is on the basal membrane or on the base of the

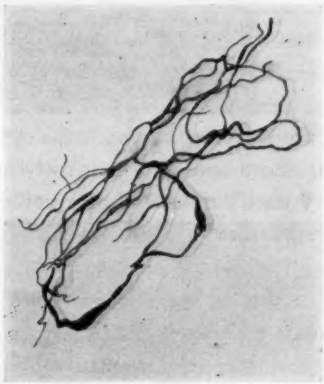
Fig. 3.



cells, because the silver method used hardly stains the thin basal membrane.

Sometimes the strong fiber forms a non-capsuled, somewhat complicated termination on the wall of the interlobular excretory duct (Fig. 4). After showing a glomerular arrangement, the fibers pass into the finnest terminal branches, being distributed on the base of the epithelium. A free hand drawing on the right side

Fig. 4.



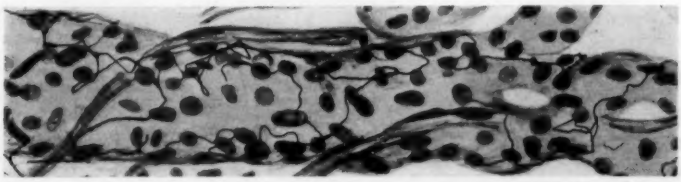
shows the fibers in the area of the photograph circumscribed by the rectangle.

2. Intraepithelial terminaton. Some fibers penetrate the basal membrane and, losing the myelin sheath, enter between the cells of the excretory duct or of the secreting portion as intraepithelial fine terminal fibers. In the wall of the thick excretory ducts such fibers are relatively abundant and their branches often arrive at the uppermost stratum of the epithelium as shown in fig. 5.

Fig. 5.

Relatively thin nerve fiber ending (*Non-reticular free nerve termination*). Coarse neural networks covering the glandular components including excretory ducts irregularly are formed as shown in fig. 6 by the branching, anastomosing and peculiar acute bending of the fibers which appear to be a little thicker than non-medullated fibers but thinner than the above described medullated fibers, before they divide into the finest terminal fibers. Although a few of the terminal fibers derived from these subepithelial relatively thin fibers distribute to the base of the cells, the majority of them enter the tissues as the intraepithelial nerve fibers.

Fig. 6.



On the secreting portion these coarse networks are well developed and the intraepithelial terminal fibers appear to be closely associated with the secretory cells, while on the large excretory duct they are scantly noticed. The finest intraepithelial fibers take a winding long course between the secretory cells, occasionally divide into branches and extend as far as the glandular lumen, as shown in fig. 7 and fig. 8. Most of them terminate sharply, though a few expand to form minute bulbous enlargements between the cells.

Though the nature of the just described fibers is still unknown, they would be regarded as sensory rather than vegetative in function, judging from their mode of distribution.

From the histological findings it is probable to assume that the terminations of the medullated thick fibers accept the impulses mainly from the basal surface of the acini and from the lumen of the excretory ducts, while the terminations of just described fibers receive stimulations chiefly from the glandular cavity and from the basal and middle strata of the wall of the excretory ducts.

Fig. 7.

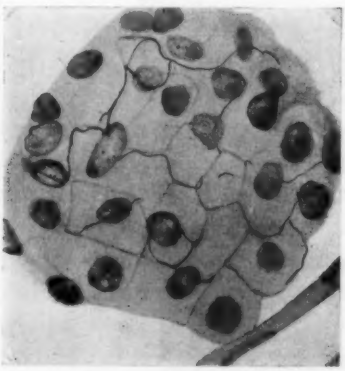
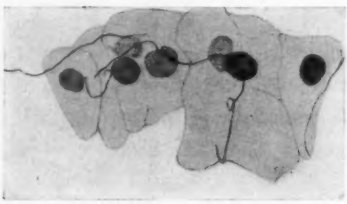


Fig. 8.



Nerve supply in the intraglandular blood vessels. The walls of the intraglandular blood vessels of all sizes are supplied with the terminal reticulum similar to what is demonstrated on the basal surface of the glandular elements. Especially, the muscular walls of the thick arteries have an abundance of them which are found not only in the perivascular connective tissue or the superficial layer of the adventitia but also in the layer between the adventitia and the media. In the perivascular connective tissue of large veins the terminal reticulum occasionally shows a peculiar anastomosis of regularly checked arrangement as photographed in fig. 9.

The strong medullated fibers and their branches taking a remarkably winding course and crossing with each other in a complicated manner are found only on the wall of the large artery and vein running along the interlobular excretory duct. Their terminal fibers penetrate the vascular wall, extend not only to tunica media but also to tunica intima and terminate sharply or bluntly as photographed in fig. 10 (vein). In the wall of the medium-sized artery and vein the unbranched termination of the medullated fiber such as those demonstrated on the secretory portion is detected.

Fig. 9.

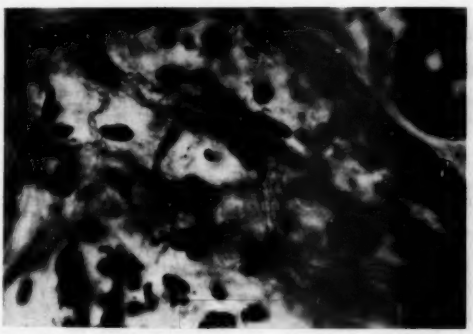
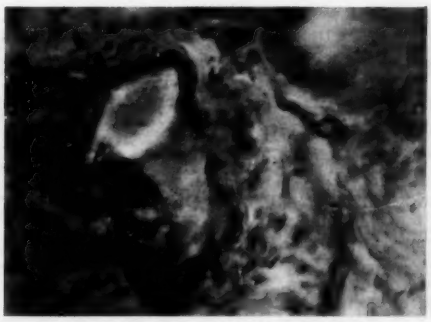


Fig. 10.



These terminations are probably to be regarded as sensory in nature, accepting the impulses from the blood stream. On small vessels including capillaries no nerve endings of this sort are recognized, although there is found the so-called vegetative terminal reticulum.

Nerve elements concerning myoepithelial cells. Myoepithelial cells are apparently innervated only by the terminal reticulum. Nerve fiber associated with protoplasm of the myoepithelial cells, such as those illustrated by Kolmer (1928) in Harderian gland of animals, was not seen in the present preparations.

Occurrence of multipolar nerve cells. In a few instances, well impregnated cells furnished with an oval nucleus and some protoplasmic processes occurred beneath the interlobular connective tissue septa. Further intensive research using suitable methods would be necessary to confirm whether the cells in question are truly of nervous nature.

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STUDIES ON THE ACTION OF THE EXTRAOCULAR MUSCLES IN MONOCULAR MOVEMENTS BY MEANS OF QUANTIFICATION OF INTEGRATED EMG

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Up to the present, the basis for the theory of ocular movements has been provided by the investigations using the topographical and solid-geometrical methods. It is evident, however, that these methods alone are not enough to make successful inquiry into the problem.

For instance, when the superior rectus contracts in the primary position, it reveals adducting action in addition to supraducting action, but when the globe is adducted we cannot know whether it contracts actively or not. The superior rectus has been generally regarded as an auxiliary adductor, however there has been no innervational evidence to support this conception.

Contraction or relaxation of the normal muscle depends on augmentation on decrementation of impulses and the electrical changes are a constant accompaniment of the innervational changes; therefore, the quantified EMG is considered to be the most suitable indicator of muscular activity.

Since 1953, Björk[1)—4)], Kuboki[5),6)], Kamouchi[7),8)], Breinin[9)—12)] and others presented the detailed reports on the EMG of the human extraocular muscles. However, the action of each extraocular muscle in ocular movements, especially in the way of quantitative measurement of action potentials, has been scarcely studied, except for Breinin's work[12)].

In the present work, the "integrated EMG" was induced from the individual eye muscle by means of the integrator, then the action of each muscle was studied in various monocular movements.

Materials and Methods

Observations were made on about 10 volunteers, all around 20 years of age, with

normal ocular motility and good vision.

The horizontal recti and the vertical muscles (recti and obliques) were studied, whereas the researches for the superior rectus and the superior oblique were abandoned, since the free movement of the eye was found to be hindered by the electrodes inserted into these muscles.

Bipolar needle electrodes with the bared tip of 2 mm. were employed, an indifferent electrode being fixed on the tragus. It was confirmed, by the preliminary experiment, that the uncoated tip of 1~1.5 mm. was enough to reveal the electrical behaviors which may represent the whole electrical activity of the muscle.

Under local anaesthesia using cocaine, the electrodes were inserted through the conjunctiva into the muscle belly. If the same integrated curve was obtained after repetition of the same eye movements it was considered that the position of electrodes remained unchanged.

Action potentials induced from the individual muscle were led to an electromyograph with push-pull type amplifier; further, one of the outputs was directly, and the other was through the integrator, led to an oscillograph of the electromagnetic type; "direct EMG wave" and "integrated EMG curve" were simultaneously recorded on a moving oscillograph-paper.

A spherical perimeter of 60 cm. in diameter was placed before the tested eye while the other eye was covered. As shown in fig. 1, eight arcs were fixed on the surface; i.e., H-H, V-V, two O-O, two h-h and two v-v. Arc H-H forms the cross line between the X-Y plane of Fick and the spherical surface of the perimeter; arc V-V, that between the Y-Z plane and the spherical surface; arc O-O, that between "Hering'sche primäre Bahnebene" and the spherical surface; arc h-h (v-v) is that between "Hering'sche sekundäre Bahnebene" and the spherical surface, and passes through the up- or down-ward 23° position (the in- or out-ward 23° position) of arc V-V (arc H-H).

A white fixation target was moved along these arcs as far as 60°, then H-H, V-V,

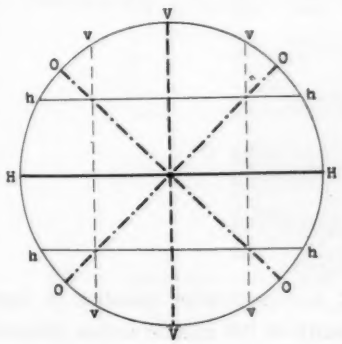

Fig. 1. Types of the monocular movements (and the planes on which the fixation line is moved).
H-H: Horizontal cardinal movement through the primary position. (X-Y plane of Fick.)
V-V: Vertical cardinal movement through the primary position. (Y-Z plane of Fick.)
O-O: Oblique movement through the primary position, i.e., Listing's rotation. (Hering'sche primäre Bahnebene.)
h-h: Horizontal movement through the vertical secondary position. (Hering'sche sekundäre Bahnebene.)
v-v: Vertical movement through the horizontal secondary position. (Hering'sche sekundäre Bahnebene.)

O-O, h-h and v-v movements were formed respectively.

The target was moved with constant velocity by the aid of a metronome, and simultaneously its posilions on the perimeter were recorded on the moving oscillograph-paper every 5° or 10°.

Integrator Unit. For the quantitative measurement of action potentials, it is inaccurate to measure simply peak-to-peak amplitudes because the wave form of EMG is ordinarily complicated. Thereupon, to measure the average amplitude of action potentials, a modified Inman's circuit[13] was designed, in which the output is proportional to the average amplitude of the input.

Its function, while related to that of a true integrator, is much nearer to the simple rectification and filtering of a complex input wave form. In principle, the circuit consists of a rectifier, an RC filter network and a balanced amplifier. The RC filter network constants were chosen so that the output of the integrator should be smooth enough to permit useful analysis, and the time delay should not affect the desired accuracy of the measurement. The suitable RC constant was 0.5 μF (fig. 2).

The height of the "integrated EMG curve" in each position is proportional to the average amplitude of the "direct EMG wave" in the same position.

Results and Discussion

Movement of the Object along the Fixaton Line (Fig. 3, 4)

In case of orthophoria, the covered eye also looks toward the object in monocular

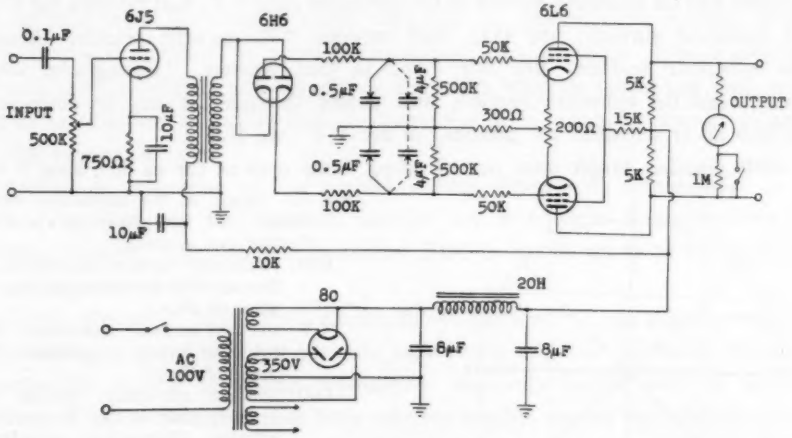

Fig. 2. Circuit diagram of integrator.

movements. This fact leads to the supposition that a considerable quantity of impulses for convergence is sent to both eyes, and the quantity of the muscle action potentials of the tested eye varies in reply to the distance to the object.

Some authors have believed, that when the fixation object approximates along the

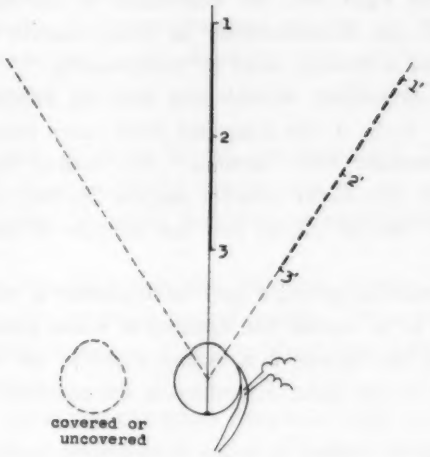

Fig. 3. Movement of the fixation object along the fixation line.
In the primary position: 1-2-3-2-1.
In the secondary position: 1′-2′-3′-2′-1′.

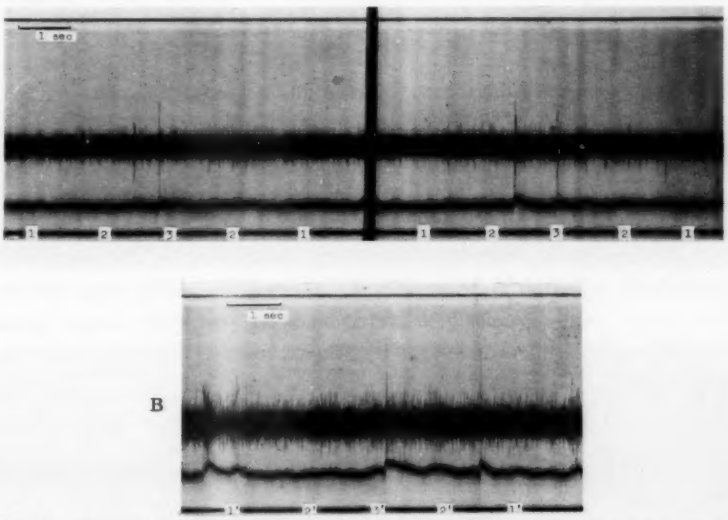

Fig. 4. Movement of the fixation object along the fixation line. Lateral rectus.
A. In the primary position (repeated).
B. In the secondary position,
In both cases the electrical activity shows no increase or decrease, and tne integrated EMG line maintains horizontality in general.

fixation axis of, e.g., the right eye, the innervation to the right medial rectus is increased by convergence and simultaneously an equal amount of innervational increase in the right lateral rectus is brought about by dextroversion.

While the present experiment showed that such an increase in innervation did not occur in both muscles. In fig. 4, the integrated EMG curve retains horizontality, and the discharge is kept at constant level. Breinin[10] also noticed this fact. Further, similar results were obtained not only in the primary position but also in the secondary and the tertiary positions. The inferior rectus and the inferior oblique also exhibited the same behavior.

Consequently, the following principle may be applicable in the monocular movements: when the fixation line is in repose, the quantity of action potentials of each extraocular muscle is independent of the distance to a fixation object or the volitional efforts etc., but depends on the position of gaze alone, regardless of the positions being primary, secondary or tertiary.

A. Horizontal recti.

Horizontal movement H-H (Fig. 5). In fig. 5, as a fixation object is moved inward from the outward 50° position (50°-out), the action potentials of the medial rectus increase little by little, and the integrated FMG curve represents no horizontal part. Beyond O°, the action potentials increase remarkedly and the integrated EMG curve ascends retaining

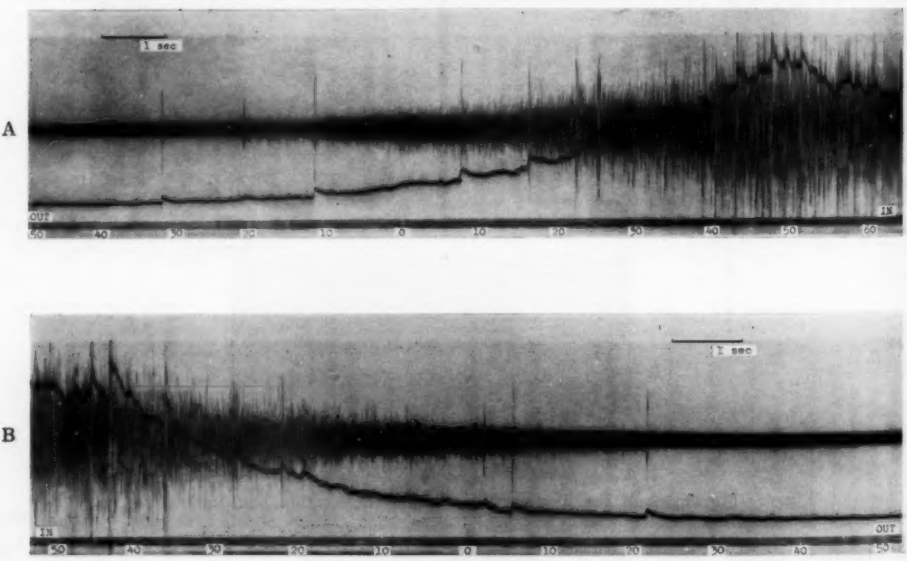

Fig. 5. H-H movement. Medial rectus.
A. "Shortening curve" obtained by the contraction.
B. "Lengthening curve" obtained by the relaxation.

a concave tendency and shows a peak just at the limit of the field of fixation ("shortening curve"). In other words, the action potentials increase no longer despite the strain to continue further rotation, but rather decrease; this may be due to the fact that the gaze returns a little toward the primary position under the burden of a maximum rotation.

In H-H movement from 50°-in to 60°-out, the integrated EMG curve of the medial rectus descends gradually along nearly the reverse course, and at the outward limit of field of fixation the quantity of action potentials is approximately zero ("lengthening curve").

Kuboki[6] and Björk[2] described that the electrical activity of the relaxing muscle never fell off to zero in the extremely rotated position. From the standpoint of the total quantity of electrical activity, however, it may be stated that it falls off to approximately zero, as described by Breinin.

Furthermore, it was found that the action potentials never increase in the extremest movement of the eye, without distinction of active or passive movement. These facts lend some support to the conception that stretch reflexes cannot be obtained from normal extraocular muscles.

Impulsiveness in so-called gliding movement (*Fig. 5,6 and others*). A slow movement of the eye without a fixation object cannot be carried out with smoothness, but in a stepping manner. A movement with a moving object, however, can be carried out with a considerable smoothness. The former is called the impulsive movement, and the latter the gliding movement.

The electromyographic aspects concerning these matters were already described by Björk and Kuboki. In the present work, however, some impulsivenesses were found even in so-called gliding movement. Repeated instantaneous bursts of discharge in the contracting muscle and similar inhibitions in the relaxing muscle are shown in fig. 4 and others, coinciding with small impulsive movements of the eye.

The impulsiveness did not vanish even though a skilled movement was obtained after repetition of the same gliding movements. Furthermore, it must be mentioned that even in the relaxing muscle there may be some bursts of discharge.

Comparison between the shortening and the lengthening curves (*Fig. 6,7*). "Shortening curve" and "lengthening curve" are generally symmetrical in respect to the shape (fig. 6), and the contracting and the relaxing muscles(the medial and the lateral rectus)are in seesaw-like relation in the aspect of electrical activity. Quantitatively, however, they are not symmetrical; the lengthening curve is turned over and superposed upon the shortening curve, then the former lies below the latter.

Fig. 7 shows this relation between the two curves, which form an area of SS'LL'; when the gaze returns outward from 40°-in without delay and similarly inward from 40°-out, S'.L and L'.S are combined into one point respectively, and a cycle SS'LL' is formed.

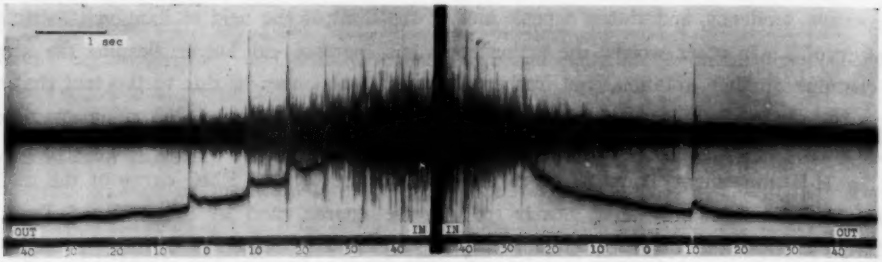

Fig. 6. Comparison between the shortening and the lengthening curves. (H-H movement. Medial rectus.)
In the same position of the eye the electrical activity of the contracting muscle is greater than that of the relaxing muscle.

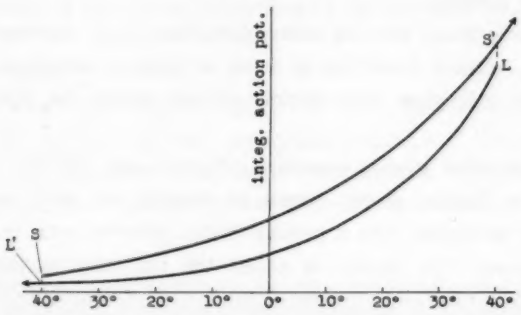

Fig. 7. Shortening curve (S-S') and lengthening curve (L-L') form a cycle by continuing contraction and relaxation.

The difference between the heights of the two curves represents the difference between the tensions of the contracting and the relaxing muscles, and this unbalance induces the rotation of the globe.

According to Bigland and Lippold[14], the electrical activity needed to lower a given load during lengthening is much less than that needed to raise it during shortening in the isotonic contraction of gastrocnemius. The relation between tension and integrated electrical activity is linear at a constant velocity of shortening and lengthening, the gradient of this line being less in lengthening. It appears of interest to see that both the extraocular muscles and the other skeletal muscles reveal the same behaviors.

Relation between shortening velocity and integrated electrical activity (*Fig. 8*). Fig. 8 shows that in the repeated H-H movement, the more the velocity of shortening increases, the more the concavity of the shortening curve is reduced, that is, the area of the cycle enlarges. While, despite an increase of the velocity of lengthening, the concavity of the lengthening curve is kept nearly constant.

Bigland and Lippold[14] stated, that at constant tension (weight), the integrated electrical activity of gastrocnemius increases linearly with the velocity of shortening, but

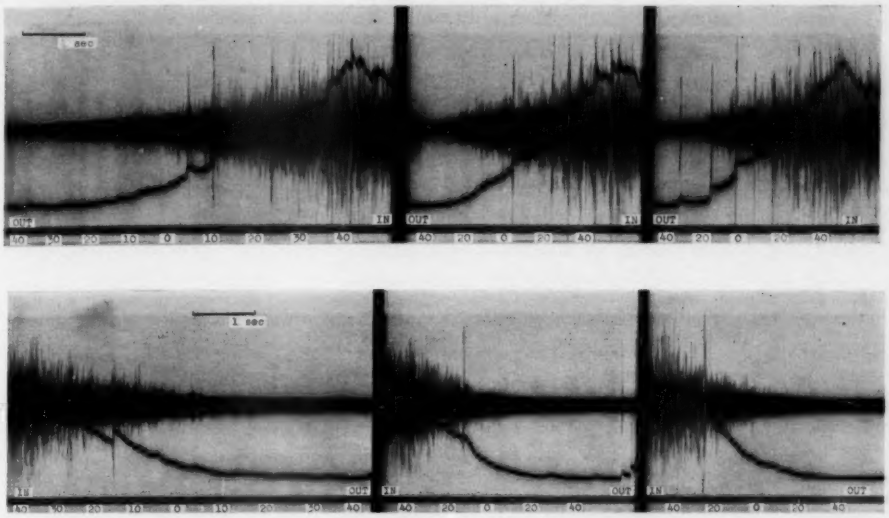

Fig. 8. The relation between the shortening (and the lengthening) velocity and the electrical activity. (H-H movement. Medial rectus.)

A. The concavity is reduced with increase of the shortening velocity.

B. No changes with increase of the lengthening velocity.

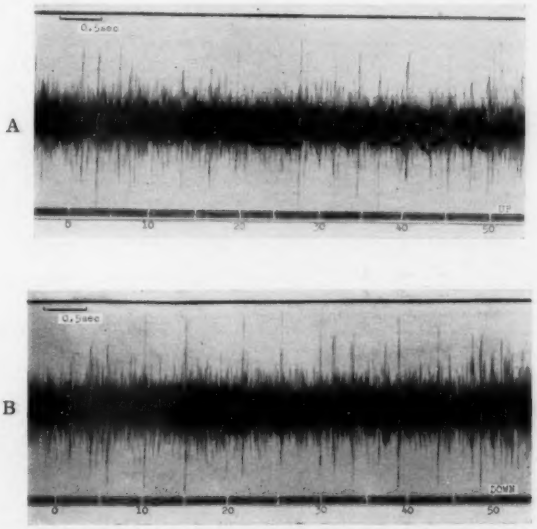

Fig. 9. V-V movement. Lateral rectus.

A. Supraduction from the primary position.

B. Infraduction from the primary position.

In both cases the electrical activity shows no increase or decrease in general.

is almost independent of velocity in lengthening. Thus, the behavior of the gastrocnemius is found to be identical with that of the ocular muscles.

The enlargement of the area of the cycle can be regarded as an increase in difference between the tensions of the contracting and the relaxing muscles.

Vertical movement, V-V (Fig. 9). When a fixation object was moved along arc V-V, the horizontal recti revealed no increase or decrease in the electrical activity in general. As shown in fig. 9, the integrated EMG curve is horizontal as a whole; viewed in parts, however, the horizontality is not rectilinear and the repeated instantaneous bursts of discharge and the similar inhibitions are interposed.

From these results, it is supposed that the two horizontal recti pull each other, retaining the constant tonus during the whole course of V-V movement, thus the eye is prevented from swerving to the right or to the left by the balance of these equal and opposing actions.

Vertical movement through the horizontal secondary position, v-v (Fig. 10, 11). The patterns of the discharge in vertical movement through the outward 23° position and through the inward 23° position are shown in fig. 10 and fig. 11 respectively.

In both cases, the electrical behaviors are similar to that in V-V movement; therefore, the similar inference may be drawn.

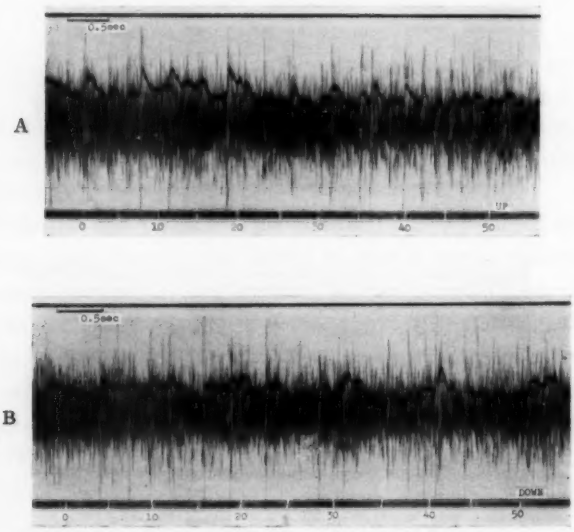

Fig. 10. v-v movement. Lateral rectus.
A. Supraduction from 23°-out.
B. Infraduction from 23°-out.
No increase or decrease of the electrical activity in general.

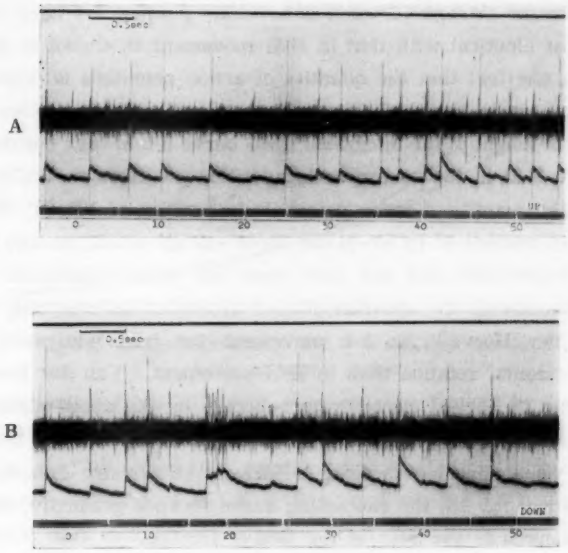

Fig. 11. v-v movement. Lateral rectus.
A. Supraduction from 23°-in.
B. Infraduction from 23°-in.
No increase or decrease of the electrical activity in general.

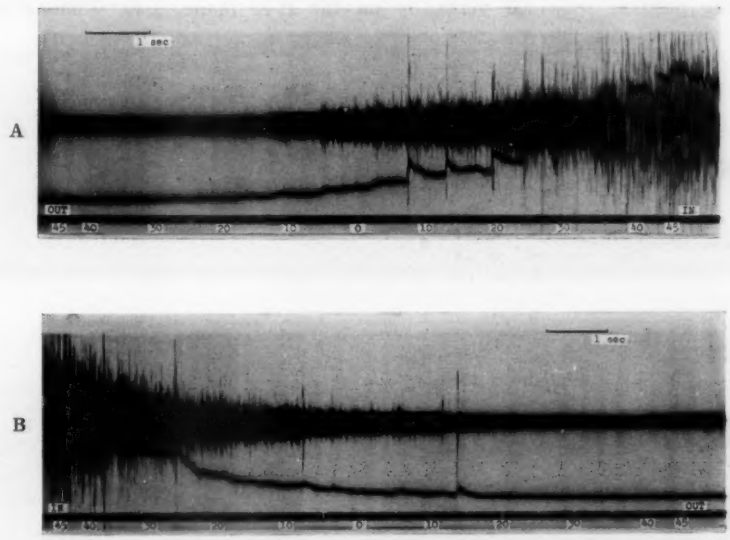

Fig. 12. h-h movement through the downward 23° position. Medial rectus.
A. Shortening curve.
B. Lengthening curve.

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Horizontal movement through the vertical secondary position, h-h (*Fig. 12*). The electrical behaviors are almost identical with that in H-H movement as shown in fig. 12.

Inferring from the fact that the quantity of action potentials is kept constant in V-V and v-v movements, it is possible to consider theoretically that when the velocity of movement is zero, the height of the integrated EMG curve h-h in each position of gaze is equal to that of the curve H-H in the corresponding position projected vertically on the horizontal plane H-H, that is, the quantity of action potentials in position of 10°·20°·30°·40°·45° on arc h-h is equal to that in position of 10°50'·21°35'·32°06'·42°21'·47°22' on arc H-H respectively.

Although the experiment did not yield such an exact numerical coincidence, the results are in agreement with the above-mentioned theoretical inference in rough estimation. According to van der Hoeve[15], in h-h movement the horizontal recti reveal a lesser efficiency of the horizontal rotation than in H-H movement. Van der Hoeve's view on the geometrical basis can be backed electromyographically by the above-stated findings.

Oblique movement, O-O (*Fig. 13, 14*). This movement corresponds to so-called Listing's rotation, and the visual line moves along "Hering'sche primäre Bahnebene" (O-O plane in fig. 1). As shown in fig. 13, the shortening curve ascends gradually, showing a concave shape, and forms a peak at the limit of the field of fixation.

The O-O curve is nearly the same as H-H curve in shape, but in height, there exists a difference between the two curves.

It should be deduced from the results in V-V and v-v movements that the quantity of action potentials in position of 10°·20°·30°·40°·50° on arc O-O is equal to that in the corresponding position projected vertically on the H-H plane, i. e., 7°06'·14°26'·22°13'·

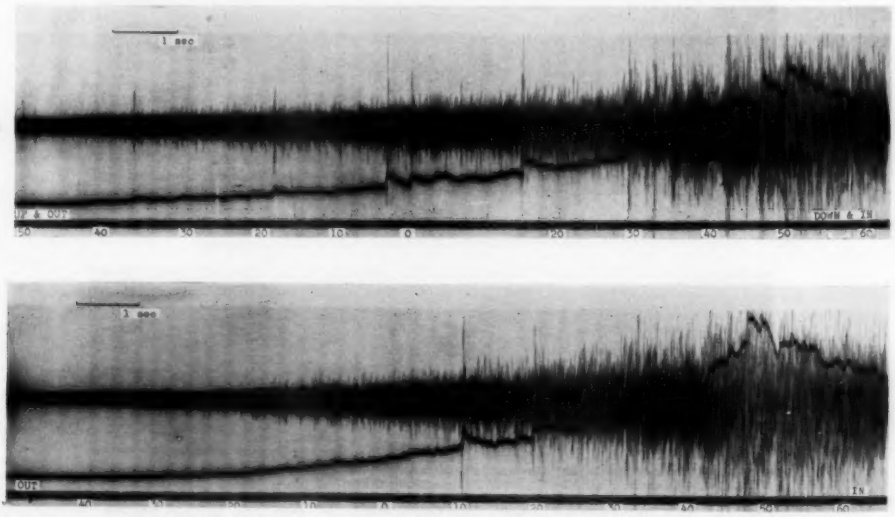

Fig. 13. Comparison between O-O movement (A) and H-H movement (B, which was carried out immediately after A). Medial rectus.

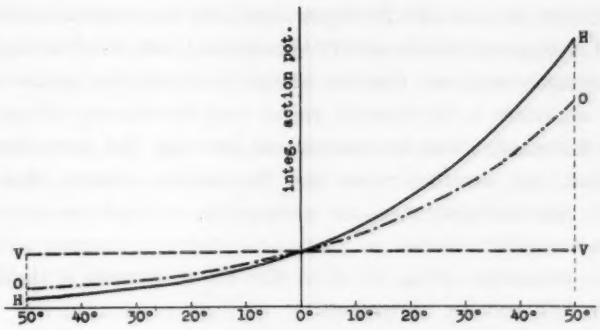

Fig. 14. Comparison among H-H, V-V and O-O curves in medial or lateral rectus.

30°41'-40°07' respectively. This inference was roughly backed by the estimation of the height of O-O and H-H curves.

The schema in fig. 14 shows the relation among O-O, H-H curves and V-V line. O-O curve lies between H-H curve and V-V line, and as the O-O plane rotates around the axis Y of Fick and changes its position from the horizontal plane (X-Y plane) to the vertical plane (Y-Z plane), O-O curve changes its shape from H-H curve to V-V line.

B. Inferior rectus and inferior oblique.

V-V movement (*Fig. 15*). The results obtained are similar to that of the horizontal recti in H-H movement.

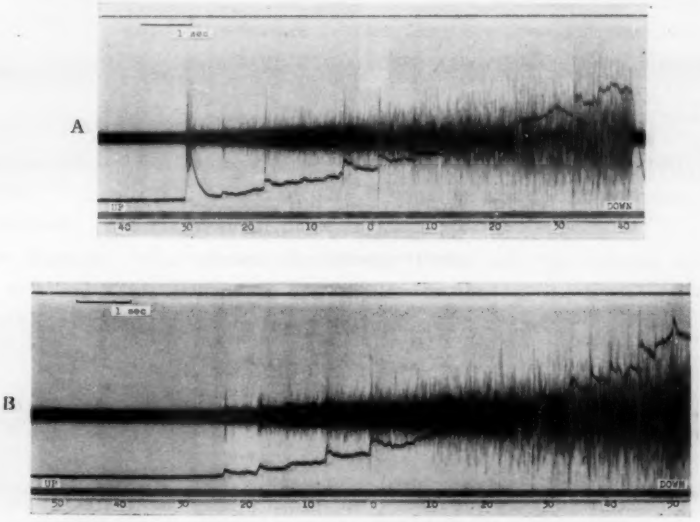

Fig. 15. Comparison v-v movement (through the outward 23° position, A) and V-V movement (B). Inferior rectus.

Since the superior and the inferior rectus (and also the superior-inferior oblique) are anatomically and solid-geometrically nearly symmetrical and the function of the two may be identical, it is easily supposed that the results in the inferior rectus (and the inferior oblique) may be applicable to the superior rectus (and the superior oblique). Accordingly, it is permissible to consider, that for instance, on elevating the globe from the extremely infraducted position, the superior rectus and the inferior oblique begin to reveal the electrical activity simultaneously and the co-operation of such an electrical activity is maintained to the end of the course.

H-H and h-h movements (*Fig. 16, 17*). The result obtained is similar to that of the horizontal recti in V-V and v-v movements.

On the basis of solid-geometrical investigation, it has been believed that the vertical recti act as active auxiliary adductors and the obliques as active auxiliary abductors in horizontal rotation of the eye. For instance, when the eye is rotated inward beyond 23°-out, the superior and the inferior rectus reinforce the adducting action of the medial rectus as auxiliary adductors. If this conception is approved, then the horizontal recti should act as the auxiliary elevators (or depressors) when the eye is rotated upward (or downward) beyond O°.

The present experiment shows that the vertical recti and the obliques maintain an almost constant level of discharge during H-H and also h-h movements; therefore, no

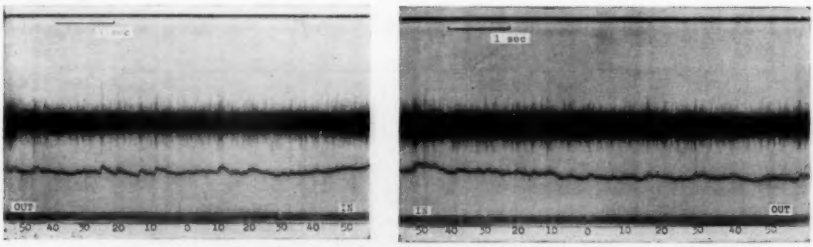

Fig. 16. Inferior rectus. H-H movement. No increase or decrease of the electrical activity in general.

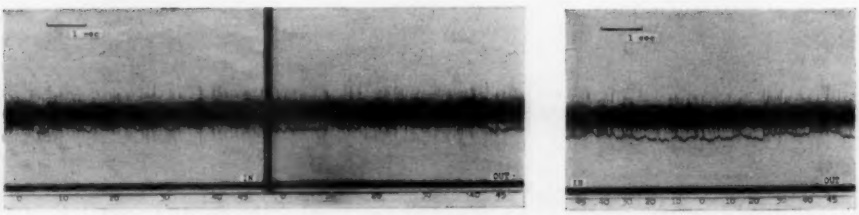

Fig. 17. Inferior oblique. h-h movement (through the upward 23° position). No increase or decrease of the electrical activity in general.

advance of active contraction or relaxation is expected. Recently Breinin[12] also described the similar findings on the vertical muscles in H-H movement pointing out the inadequacy of long-maintained concepts that the vertical muscles are active accessory horizontal-rotators.

v-v and O-O movement (*Fig. 15*). The results obtained are similar to that of the horizontal recti in h-h and O-O movements respectively. As stated in the section of horizontal recti, the quantity of action potentials in each position is nearly equal to that of the V-V curve in the corresponding position projected horizontally on the V-V plane.

It is interesting to note that the electrical behaviors of the inferior rectus are identical in both v-v movements through 23°-in and 23°-out, despite the fact that the action of the vertical component of the inferior rectus is greater in the latter than in the former.

Summary

Quantitative measurements of action potentials were made using the integrator. Action potentials were induced by bipolar needle electrodes with bared tip of 2 mm., and "direct EMG wave" and "integrated EMG curve" were simultaneously recorded.

1. The theory is presented that the quantity of innervation to each extraocular muscle is a function of the position of gaze and the moving velocity of the fixation line; when the fixation line is in repose it is a function of the position of the eye alone.

2. In H-H movement, "shortening curve" and "lengthening curve" (both are integrated EMG curves) of the horizontal recti were obtained. The shortening curve forms a peak at the limit of the field of fixation, that is, the action potentials increase no longer despite the strain to continue further rotation.

3. In V-V and v-v movements, the horizontal recti did not reveal any increase or decrease in the electrical activity as a whole.

4. In h-h movement and also in O-O movement, the quantity of action potentials of the horizontal recti in each position of gaze is equal to that in the corresponding position projected vertically on the horizontal plane H-H.

5. The electrical behaviors of the inferior rectus and the inferior oblique in H-H, h-h, V-V, v-v and O-O movements are similar to that of the horizontal recti in V-V, v-v, H-H, h-h and O-O movements respectively.

6. In horizontal movements (H-H and h-h) of the eye, one of the horizontal recti contracts and the other is relaxed, while all of the vertical muscles (recti and obliques) maintain a constant level of discharge and never receive any increase in innervation. In vertical movements (V-V and v-v), two of the vertical muscles contract and the other two are relaxed, and the electrical activity of the horizontal recti remains unchanged. In oblique movements, three muscles co-contract and the remaining three are relaxed.

(Published in Acta O. Jap. 61:1570, 1957 in Japanese.)

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A CLINICAL STUDY ON BEHÇET'S SYNDROME

I. OCCURRENCE RATES OF THIS SYNDROME IN ENDOGENOUS UVEITIS

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Only few statistics on iritis, iridocyclitis or uveitis have been reported in this country and foreign countries. Etiological analysis of uveitis has become more accurate due to the advancement of the therapeutic trial test and other diagnostic means, and naturally some changes must be expected in these statistics.

The relationship between uveitis and the mucocutaneous ocular syndrome was emphasized in the previous paper[1]. Five hundred and twelve outpatients in Tokyo University Hospital were investigated from this viewpoint and the presence of relationship was confirmed. This paper involves the statistical analysis of Behçet's syndrome and other kind of uveitis.

Method

Thirty eight thousand and fifty three outpatients who have visited our clinic in the last three years between 1954-1956 were investigated. (40 patients were excluded for incomplete records.)

The following points were taken into consideration in making the statistics:

1) Patients with primary or endogenous uveitis, including iritis and iridocyclitis, were selected. Those with Harada's disease, Vogt-Koyanagi's disease and sympathetic ophthalmia were excluded, as in other reports on this subject.

2) The diagnosis of uveitis was made not only on the examination at the first visit

but also on the symptoms developed afterwards.

3) One patient suffering either on one eye or both eyes was counted as one case in the statistics.

4) Fiducial level of percentage is decided 98%. Most of the statistical analysis was made by the X^2-test.

The Statistics on Uveitis

I. Occurrence rate of uveitis. As shown in Table 1, 512 of 38053 patients studied (1.35-1.21%) were found to have uveitis. The X^2-test proved, that there is no significant difference between the annual occurrence rates ($p<0.01$).

In this country, percentage of uveitis against all eye diseases were about 1%: it was 1.7% (R. Imai, 1934-5[2]) and in our clinic, it was 1.23% in 1921, 0.85% in 1935, 0.89% in 1942 and 0.94% in 1955. (M. Mitarai[3], K. Ishiwara[4] and Y. Uchida[5].)

Table 1. Occurrence Rate of Uveitis

year	number of cases studied	uveitis	
		total	percentage ($p<0.02$)
1954	11994	176	1.84~1.14%
1955	12868	187	1.82~1.14%
1956	13191	149	1.42~1.21%
total	38053	512	1.35~1.21%

The present result thus agrees with those of other investigators.

II. Difference in sex, age and side of the diseased eye. (Table 2 and 3)

Sex. Morbidity of male was 1.34-0.97% (248 of 21444) and that of female was 1.84-1.31% (264 of 16609). The X^2-test proved that morbidity in the female was higher than that in the male ($p>0.01$). This result agrees with those of W. Gilbert[6] but not with

Table 2. Sex and Age Difference in $\frac{\text{Cases of Uveitis}}{\text{Number of Cases Studied}}$

sex \ age	0-	10-	20-	30-	40-	50-	60-	70-	80-	total	per centage ($p<0.02$)
male	6/2038	30/4439	77/6651	63/2349	37/2187	21/2037	9/1344	5/361	0/38	248/21444	1.34~0.97%
female	5/1723	26/3197	69/4216	64/2120	45/1848	37/1912	15/1231	3/355	0/25	264/16609	1.84~1.31%
total	11/3761	56/7618	146/10867	127/4469	82/4035	58/3949	24/2575	8/716	0/63	512/38053	
percentage ($p<0.02$)	0.57~0.12%	1.02~0.51%	1.69~1.05%	3.56~2.23%	2.78~1.53%	2.52~1.03%	1.47~0.54%	2.47~0.40%	0		1.35~1.21%

Table 3. Side of Diseased Eye

diseased eye		total		percentage ($p<0.02$)
bilateral		242		53.30～42.41%
unilateral	right	137	264	57.59～46.70%
	left	127		((right)60.17～43.50%) ((left)56.50～39.84%)
unknown*		6		

*: Infants and other cases in which the examination by biomicroscopy are not possible.

Imai's. In these reports, the number of male and female with uveitis was simply compared, whereas the whole number of the patients examined was not taken into consideration. Therefore these perecntages are not reliable.

Age. Uveitis was found mostly at 30-49 years of age and rare at 0-19 years. R. Imai and W. Gilbert also reported the similar tendency.

It is of interest to see, that most of the uveitis that occurred in the aged group of 60's and 70's showed an extremely mild serous form which is cured with only atropin instillation.

Side of the diseased eye. The side of the diseased eye is shown in Table 3. In the occurrence of uveitis, no significant difference could be found between bilateral and unilateral or betwen the right and the left.

Discussion on the etiology of uveitis

I. Uveitis with known etiology. (Table 4 and 5) It is difficult to determine the etiology of uveitis since histological, bacteriological or serological examination of this organ itself and the substances around this organ is almost impossible in living persons.

Various diseases shown in Table 4 have been considered as the cause of uveitis, but the true pathogenesis are not proved completely. Recently skin test and cytopathological examination of the aqueous humor have been used in some European and American countries but it is difficult to apply these method to all clinical cases.

Therefore, the author made a criterion of the underlying disease as follows.

Tubercular. On clinical findings, tubercular uveitis was suspected, of which reacted to chemotherapy of 2-3 weeks with Streptomycin, PAS or INAH was diagnosed as tubercular. History, general physical condition, chest X.ray picture and Pirquet's reaction were taken as reference. Focal reaction with subcutaneous injection of tuberculin and Middlebrook-Dubos' reaction of aqueous humor were neglected[7].

Syphilitic. Serological thests of syphilis were done by Ogata's test, aggultinin test and slide test. The patients with positive result were classified as syphilitic.

Rheumatic and diabetic. In general rheumatism and diabetes mellitus are considered to be related with the mucocutaneous ocular syndrome[9]. Uveitis found in patients with arthritis and musclar disorder due to rheumatism were classfied as rheumatic. Diabetic

Table 4. Underlying Diseases Considered as Causes of Uveitis

granulomatous inflammations
  tuberculosis
  syphilis
  sarcoid
  leprosy etc.
acute febrile diseases
  influenza
  Weil's disease etc.
chronic pyogenic diseases
  tonsillitis
  sinuitis etc.
metabolic disorders
  diabetes mellitus
  gout etc.
rheumatism, heterochromia etc.

Table 5. Uveitis with Apparent Causes.

reporter	total number of uveitis	uveitis with apparent causes						uveitis with unknown etiology
		tubercular	syphilitic	rheumatic	diabetic	others	total	
M. Straub 1907-9	161	69 42.9%**	37 23.0%**	3 1.9%**	1 0.6%**	6 3.7%**	116 72.1%**	45* 27.9%**
W. Gilbert 1930	500	228 45.6%	83 16.6%	15 3.0%	4*** 0.8%	83 16.6%	413 82.6%	87 17.4%
R. Imai etc. 1934-5	215	99 46.04%	67 31.16%	2 0.93%	0 0	6 2.79%	174 82.92%	41 19.07%
T. Kato 1954-6	512	33 6.4%	28 5.5%	4 0.8%	4 0.8%	6 1.2%	75 14.7% (19.36~11.40%)	437 85.7% (88.60~80.64%)

*: including "Iritis recidivans"
**: calculated by the author
***: including "Flunculosis"

uveitis was diagnosed in the same manner.

Others. Other cases with apparent diseases such as febrile sickness (Weil's disease etc.) and sarcoidosis were classified in this group. In Table 5, 512 cases of uveitis were classified by these criteria and the statistics made by M. Straub[9], W. Gilbert and R. Imai are also tabulated.

Only 75 cases of 512 or 19.36-11.40% have some known etiology, therefore the rest (88.60-80.64%) must be classified as the cases with unknown etiology. It is noted in the present results that the number of tubercular and syphilitic uveitis has markedly decreased, while that of the cases with unknown etiology increased, as compared with statistics compiled by M. Straub and others. The discrepancy may be due to the fact that

infectious diseases like tuberculosis and syphillis have diminished or have been alleviated with the remarkable development of chemotherapy, and that therapeutic trial test was chosen as the best diagnostic method for tuberculosis. A similar tendency was observed in the report of A.C. Woods[10], the importance of toxoplasmosis being emphasized.

It is likely, that toxoplasmosis are included in the cases with unknown etiology in my statistics; toxoplasmosis is being investigated by our collegues.

II. Uveitis with unknown etiology. (Table 6)

Since H. Behçet[11] reported in 1937 that uveitis in the form of "Uveitis recidivans cum hypopyone" is accompanied with symptoms of probably allergic reaction in the skin and mucous membrane, Reiter's disease, Stevens-Johnson's disease and many similar diseases have been involved in the mucocutaneous syndrome. The true etiology of all these diseases is still unknown despite of many studies. The author wishes to emphasize, that a large number of uveitis is the manifestation of this syndrome.

The "complete type" has symptoms both on the skin (erythema nodosum, erythema exudativum multiforme etc.) and on the mucous membrane (aphthous stomatitis, genital ulcer etc.), while the "incomplete type" shows symptoms either on the skin or on the mucous membrane. Classifing uveitis in this manner, an astonishing fact was discovered, that is uveitis including "incomplete type" amounts to 77 of 437 cases (23.02-13.82%) of unknown etiology, as shown in Table 6.

Table 6. Mucocutaneous Ocular Syndrome

year	uveitis with unknown etiology	mucocutaneous ocular syndrome		
		complete type	incomplete type	total
1954	149	8	16	24
1955	156	17	11	28
1956	132	11	14	25
	432	36	41	77
percentage to uveitis with unknown etiology ($p<0.02$)		11.73~ 5.53%	13.25~ 6.48%	23.02~ 13.82%
percentage to all cases of uveitis ($p<0.02$)		10.07~ 4.70%	11.38~ 5.50%	19.84~ 11.72%

Conclusion and Summary

Thirty eight thousand and fifty three outpatients who have visited our clinic in the last three years between 1954-1956 were examined and the following results were obtained.

1. There were 512 cases or 1.35-1.21% ($p<0.02$) of endogenous uveitis. Above the half of the cases was found in the female. Occurrence rate was highest in the thirties and forties, lowest in children below ten years of age, and those of other age-ranges did

not statistically differ from each other. No significant differences were present between the occurrence rate of bilateral and unilateral cases, or between those of right and left eyes.

2. Of 512 cases, only 75 cases or 19.4-11.4% ($p<0.02$) were considered to have apparent etiological causes such as tuberculosis, syphilis etc., while 437 cases or 88.6-80.6% ($p<0.02$) had no apparent causes.

3. Uveitis of mucocutaneous ocular syndrome, complete and incomplete type altogether, was observed in 77 cases of 437 cases with unknown etiology (23.1-13.8%, $p<0.02$) or 16.9-11.7% ($p<0.02$) of all 512 cases.

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METABOLISM IN INNER AND OUTER LAYERS OF THE RETINA

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Introduction

In the retina, not only the oxygen consumption but aerobic glycolysis are very intensive. What kind of mechanism causes this intensive and specific tissue metabolism? In what layer of the retina does it act? These questions are the most interesting and important ones for the purpose of investigating the problem of visual sense. As the layer, in which the active metabolism is carried out the visual cell layer is naturally noticed, according to mechanism and structure of the retina, and Kimura, Hanawa, Hubbard and others have already measured the respiration of outer segments of rods. At present time, however, it is still impossible to separate exactly each layer of the retina and to measure respectively its tissue respiration. Noticing that the inner layer is chiefly nourished by the central artery and the outer layer by the ciliary arteries, we judged that we would be able to estimate the value of metabolism held mainly in the outer layer of the retina if we measured it after the ligation of the central artery and the value of inner layer after the ligation of ciliary arteries, and practiced to measure the tissue metabolism in the respective cases.

Several years ago, Takehisa Oguchi, on the similar standpoint, measured the tissue metabolism of the retina after one hour from the ligation of central and ciliary vessels, and found that the Qo_2 of the retina after the ligation of central vessels was decreased in 45.3% and the $Q_M^{O_2}$ was decreased in 14.2%, and that the Qo_2 after the ligation of ciliary vessels was decreased in 56.2% and the $Q_M^{O_2}$ was decreased in 31.8%. Then, he considered that the respiration of the retina was related to the intactness of nuclear layer, and that the oxgen consumption in the inner and outer layer of the retina were almost equal to each other, or that of the outer layer was slightly larger. Judging the fact that the aerobic glycolysis was more influenced by the ligation of ciliary vessels, he regarded the relation of it and the function of visual cells as important. In our experiment, in the same way, however, the Qo_2 and $Q_M^{O_2}$ of the retina after one hour from the ligation of ciliary vessels were strikingly decreased, respectively in 52.0% and 40.1%, while in the cases of the ligation of central vessels the values of tissue metabolism had not great changes, compared with those in the control cases. We also supposed at first that the tissue metabolism would be reduced, because after the ligation of central vessels we could observe some histopathological changes in the inner layer of the retina, but we got the contrary results to it. Then, we investigated cautiously and repeatedly theses matters, but we could not observe any remarkable decrease. In spite of the fact that the inner layer of the retina had some pathological changes, the value of tissue metabolism was near to the normal value, unless there was some change in the outer layer. And in the retina which had some destroyed findings, caused by the ligation of ciliary vessels, in the outer layer, the Qo_2 and $Q_M^{O_2}$ showed the strikingly decrease values. From these facts, it can be considered that the values of Qo_2 and $Q_M^{O_2}$ of the normal retina measured by Warburg's manometric method, are mostly originated to the function of the outer layer of the retina.

Methods

As the experimental animals, healthy adult white rabbits were employed. At the ligation of central vessels of the retina the insertion of the tendon of M. rectus superior and bulbar conjunctiva were grasped with a hooked pincette and passed with a suture thread. Behind this position the bulbar conjunctiva was cut and stripped out. The M. rectus superior was cut down and when the dissection reached to the posterior pole of globe, without giving any injury of vessels, the optic nerve was exposed. Under the optic nerve, with a small arterial needle and unhooked pincette a suture thread was passed and it was closely ligated, as nearer to the globe as possible. After the ligation it was confirmed by the ophthalmoscopy that the blood stream of the central artery was completely stopped.

At the ligation of ciliary vessels, after the cutting of M. rectus superior, same to the case of central artery, the bulbar conjunctiva was dessected all round and the other ocular muscles were all cut down. To the posterior pole of globe, all attached tissues were cautiously dissected. After that, under the optic nerve a suture thread was passed, similarly to the case of central vessels, and it was turned to the under side of globe and was closely

ligated. After the ligation, the fact that the central artery never received any injury, was confirmed by the ophthalmoscopy.

The retina was cut off by the following method. Without narcosis the globe was rapidly extirpated and cut off at the position, some what posterior to its equator. Next, in Ringer's solution the retina of posterior half of globe was cut down into halves with choroid and sclera, and when they were slightly shaked in the solution the retina was easily loosed. Then, without directly grasping the retina with pincette and without attaching pieces of the vitreous to it, it was taken out cautiously.

For the purpose of measuring the tissue metabolism of the retina, Warburg's manometer was used, and as the tissue floating liquid, Ringer's solution was applied. The temperature at measurement was 37.5°C. The time from the extirpation to the first reading of scale was about 40 minutes. The preliminary shaking could be shortened by adequately warming Ringer's solution forhand.

The quantitative analysis of thiamine depended upon the thiochrome method. And for the purpose of the measurement of esterification ability of thiamine, to the tissue floating ilquid thiamine was given in the required concentration, and after shaking for 1.5 hours at the illumination of 120 lux on liquid surface and 37.5°C temperature, the quantity of thiamin was measured. And from the difference of both the thiamine quantities, the esterification ability was calculated.

Results

The results of measurement of tissue metabolism are showed in Table 1 (a) and 1 (b). After one hour from the ligation of central vessels of retina, the Q_{O_2} and $Q_M^{O_2}$ had no great change, but contrarily after one hour from the ligation of ciliary vessels the Q_{O_2} was decreased in 52% and the $Q_M^{O_2}$ in 40.1%. The values in Table 1 (b) were the results of one individual which received the ligation of central vessels in one eye and of ciliary vessels in the contralateral eye.

The quantity of thiamine in the retina is showed in Table 2. In consideration of the fact that after the ligation of ciliary vessels the wet weight was increased, it was supposed that the quantity of thiamine at that time had no remarkable change. But at the case of the ligation of central vessels the total quantity of thiamine was decreased in about 20%.

The esterification ability of thiamine in the retina at the case when the giving concentration to the floating liquid was 0.1γ/cc, was showed in Table 3.

Discussion

As already known, the changes of Q_{O_2} and $Q_M^{O_2}$ had some differences, according to the grade of tissue damage. The changes of the inner layer of the retina caused by the ligation of ciliary vessels appeared more rapidly and were more intensive, than those by the ligation of central vessels. After the stoppage of blood stream, the decomposition of protein caused by autolysis is to appear. According to Ichikawa, who investigated histo-

Table 1 (a). Tissue Metabolism of the Retina After One Hour from the Ligation of Alimentary Vessels of the Retina

Rabbit			Eye with Ligation of Central Vessels			Control		
No.	Sex	Body Weight (g)	Dried Weight(mg.)	Qo_2	$Q^O_{M_2}$	Dried Weight(mg.)	Qo_2	$Q^O_{M_2}$
1	♀	1780	4.457	−15.2	+32.2	4.549	−15.6	+32.4
2	♀	2020	5.881	−16.2	+27.1	5.411	−16.7	+29.4
3	♂	1920	4.456	−17.9	+29.0	4.895	−17.8	+30.1
4	♀	2770	5.163	−17.0	+28.6	5.448	−17.1	+30.7
5	♀	2430	5.253	−18.6	+33.5	6.349	−18.4	+33.5
6	♂	1570	5.257	−18.2	+27.0	5.008	−18.5	+32.5
7	♂	1480	4.684	−17.2	+30.0	5.040	−18.0	+28.4
8	♀	1950	4.416	−15.9	+33.4	4.648	−15.0	+32.4
Average				−17.0	+30.1		−17.1	+31.1

Rabbit			Eye with Ligation of Ciliary Vessels			Control		
No.	Sex	Body Weight (g)	Dried Weight(mg.)	Qo_2	$Q^O_{M_2}$	Dried Weight(mg.)	Qo_2	$Q^O_{M_2}$
9	♂	1360	4.994	−9.2	+17.1	4.303	−17.0	+29.1
10	♀	1950	4.265	−7.8	+19.0	3.963	−17.8	+30.5
11	♂	1750	4.556	−8.2	+19.3	4.678	−17.9	+31.5
12	♀	2000	3.998	−8.2	+20.4	4.524	−16.5	+33.2
13	♀	1900	3.632	−8.1	+16.6	3.881	−17.2	+30.1
Average				−8.3	+18.5		−17.3	+30.9

Table 1 (b). Tissue Metabolism of the Retina After One Hour from the Ligation of Alimentary Vessels of the Retina

Rabbit			Eye with Ligation of Central Vessels			Eye with Ligation of Ciliary Vessels		
No.	Sex	Body Weight (g)	Dried Weight(mg.)	Qo_2	$Q^O_{M_2}$	Dried Weight(mg.)	Qo_2	$Q^O_{M_2}$
14	♂	1300	4.069	−17.0	+32.6	3.692	−6.9	+19.3
15	♂	1890	5.086	−18.6	+30.2	5.038	−8.5	+19.1
Average				−17.8	+31.4		−7.7	+19.2

Table 2. Thiamine Quantity in the Retina After One Hour from the Ligation of Alimentary Vessels of the Retina

Thiamine Quantity	Normal					After Ligation of Central Vessels					After Ligation of Ciliary Vessels				
	No.	Body Weight (g)	Wet Weight of Retina (mg.)	Retina (γ%)	Blood (γ/dl.)	No.	Body Weight (g)	Wet Weight of Retina (mg.)	Retina (γ%)	Blood (γ/dl.)	No.	Body Weight (g)	Wet Weight of Retina (mg.)	Retina (γ%)	Blood (γ/dl.)
Total	16	1920	67.5	122	12.5	22	2020	61.0	92	20.8	16	1920	67.0	106	12.5
	17	1900	56.0	132	14.5	23	2040	61.5	92	14.1	17	1900	54.0	108	14.5
	18	2000	128.0	133	19.6	24	2600	60.0	102	15.3	26	1650	133.5	95	18.5
	19	1930	115.0	127	17.3	25	1700	53.0	110	17.3	27	1800	135.0	110	17.7
	20	1700	95.0	125	17.0						28	1940	162.0	110	20.4
	21	2290	139.0	135	18.0										
	22	2020	56.0	119	20.8										
	23	2040	61.5	126	14.1										
	24	2600	68.0	125	15.3										
		Average		127.1	16.6		Average		99.0	16.9		Average		105.8	16.7
Free Type	29	2000	141.0	10	14.1	33	1700	57.5	8	12.2	35	1770	63.0	10	11.3
	30	2100	136.5	9	17.3	34	1790	58.0	8	15.3	36	1700	77.0	7	13.6
	31	1850	112.0	9	15.3	35	1770	57.0	8	11.3	37	2140	68.0	7	15.4
	32	1720	110.5	9	13.7	36	1700	65.0	10	13.6	38	2140	71.0	7	15.4
		Average		9.3	15.1		Average		8.5	13.3		Average		7.8	13.9

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Table 3. Esterification Ability of Thiamine of the Retina After One Hour from the Ligation of Alimentary Vessels of the Retina (Thiamine: 0.1γ/cc.)

Total Thiamine Quantity in Liquid	Normal					After Ligation of Central Vessels					After Ligation of Ciliary Vessels				
	No.	Body Weight (g)	Dried Weight of Retina (mg.)	Esterificated Thiamine Quantity in Amount (γ)	Esterificated Thiamine Quantity pro mg. (γ)	No.	Body Weight (g)	Dried Weight of Retina (mg.)	Esterificated Thiamine Quantity in Amount (γ)	Esterificated Thiamine Quantity pro mg. (γ)	No.	Body Weight (g)	Dried Weight of Retina (mg.)	Esterificated Thiamine Quantity in Amount (γ)	Esterificated Thiamine Quantity pro mg. (γ)
1.2 γ	46	2360	5.691	0.20	0.04	55	1600	4.069	0.19	0.05	55	1600	3.692	0.05	0.01
	47	1800	6.316	0.46	0.07	56	1870	5.086	0.19	0.04	56	1870	5.038	0.05	0.01
	48	2260	5.221	0.20	0.04	57	1570	5.257	0.19	0.04	59	2700	4.524	0.05	0.01
	49	2010	4.495	0.12	0.03	58	1600	4.684	0.16	0.03	60	1900	3.887	0.05	0.01
		Average		0.25	0.05		Average		0.18	0.04		Average		0.05	0.01
0.4 γ	46	2360	5.948	0.21	0.04	55	1600	3.589	0.11	0.03	55	1600	3.326	0.04	0.012
	47	1800	6.309	0.17	0.03	56	1870	4.270	0.06	0.01	56	1870	4.076	0.01	0.003
	48	2260	4.962	0.16	0.03	57	1570	4.269	0.09	0.02	59	2700	3.759	0.02	0.005
	49	2010	4.025	0.09	0.02	58	1600	4.603	0.05	0.01	60	1900	4.000	0.03	0.008
		Average		0.16	0.03		Average		0.08	0.02		Average		—0.03	0.007

chemically the nucleic acids in the retina of died mice, the RNA of protoplasm was decreased most rapidly in the inner segment of visual cells and most slowly in the layer of ganglion cells. These facts are considered to show that RNA in the visual cells is unstable, compared with that in the other cells of impulse conducting system, because in the former regions the metabolism is more active.

The values of tissue metabolism of the retina after one hour from the ligation of central or ciliary vessels, are not considered to show completely the values of tissue metabolism of the inner or outer layer of the retina. For instance, it is reasonable to suppose that various kinds of decomposed products of degenerated tissues are to be desolved into the floating liquid, and that they may have some influences to the values of the quotients. Even in consideration of these factors, however, as showed in the experimental results of the authors, it can be asserted that the values of tissue metabolism are small in the inner layer and extremely larger in the outer layer.

The tissue metabolism of the cornea and the crystalline lens is larger in embryonal stadium than in postnatal time. In the retina of a human embryo of 7 months old, which K. Saito could observe, $Q_{O_2} = -9.2$ and $Q_M^{O_2} = +10.7$. And in that of an adult $Q_{O_2} = +18.2$ and $Q_M^{O_2} = +19.9$. As to the reason that the values of tissue metabolism are so small, in spite of the numerousness of nucleus and of the period during development, we would point out that the visual cells do not yet act in the embryonal retina.

Judging from the thiamine demand in the retina, it had no great difference to the normal value, even at the case after the ligation of ciliary vessels. This fact can be easily explained when we presume that the spending of thiamine was stopped due to the rapid decrease of vital power of the outer layer of the retina, mainly of visual cells. The ratio of esterification ability of thiamine between the inner and outer layers of the retina, as showed in the tables, can be presumed to be 1 : 4 or 1 : 3. Therefore, it can be said that the metabolism in the outer layer of the retina is more active than in the inner layer.

Then, we attach great importance to the part of the outer layer of the retina, as a reason that the retina has specific values of tissue metabolism, compared with the other tissues. And we also suppose that the values of all tissue metabolism of the retina can be almost determined by the values of outer layer of the retina.

Conclusion

By means of the Warburg's indirect method, the authors measured the tissue metabolism of the retina in white rabbits, after one hour from the ligation of central or ciliary vessels of the retina. As the results, in case of the ligation of central vessels, $Q_{O_2} = -17.0$ and $Q_M^{O_2} = +30.1$; these had no great difference to the control case, only a small decrease respectively of 3.4% and 3.2%. But in case of the ligation of ciliary vessels, $Q_{O_2} = -8.3$ and $Q_M^{O_2} = +18.5$. These are respectively the decrease of 52% and 40.1%, compared with the control case.

As for the esterification ability of thiamine in the retina, the ratio between the outer

and inner layer is considered 1 : 3—4. And the demand of thiamine is larger in the outer layer.

The above mentioned facts show that the metabolism in the outer layer of the retina is more active than in the inner layer. It can be concluded that this is closely connected to the function, "to see".

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EXPERIMENTAL STUDIES ON TWILIGHT VISION AND ROAD SAFETY

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Relative to studies on twilight vision and road safety, the problems of head light and wind shields call for special consideration, as an exposure to powerful head light lowers the subsequent visual performance and leads to the occurrence of traffic accidents. Regarding exposure to head lights the following three problems receive attention, namely, first that exposure to head lights is instantaneous, that the environmental illumination intensity before exposure may influence greatly the subsequent visual performance, and if such an influence occurs what environmental illumination intensity will render the eye after exposure to see with ease and hence prevent accidents and help to maintain traffic safety. Secondly, there arises the problem of after image resulting from exposure to powerful light. When during disappearance of the positive and negative after images there appears the succeeding colored light test object, the color of the test object may not be properly discerned. Such is believed to influence greatly false perception of traffic signals, and thus present an important problem in traffic safety. Thirdly, consideration of the effects of tinted wind shields has to be made, namely how difficult will tinted wind shields render perception of test objects with poor contrast, and in connection with this how can the glare resulting from exposure to head light be lowered.

The above were investigated under various experimental conditions, such as examining dynamically by an electronic counter the relationship between after image and visual performance after exposure to powerful light under various twilight conditions. Experiments were further conducted to find what the effects on the above are of tinted wind shields.

Method

The examinee was subjected to twilight adaptation under an various environmental illumination intensities. With start of the experiments the examiner puts on a double switch when the flat light for exposure is lighted and the relay begins to move. After a definite time has elasped the flat light is put out by the relay, and the current flows into the electronic counter. Simultaneous with the switching out of the light the examinee is made to gaze into the test surface of Nagel's adaptometer or a monochrometer of a "Hitachi" spectrophotometer, and when the stimulus light is recognized, the key attached directly to the electronic counter (Yamakoshi Seisakusho Made) is pressed when the counter comes to a stop. The time that elapses up to the first perception of stimulus light after exposure will then be shown accuratly, in 1/1000 of a second. This is read and recorded by the examinee. The writer under-took the role of examinee, and with emmetropia of both eyes and the left eye, the environmental illumination intensities were measured by means of a photometer placed in front of the eyes. In experiments on the influences of tinted wind shields, Matsuda's colored filters, IRO-1 and S-T1, were used.

Experimental conditions and Results

1. Visual performance for white light objects

1) Influence of environmental illumination intensity on visual performance for white light objects after exposure

As conditions three grades of environmental illumination intensity, namely darkness, 50 lx and 200 lx, were employed, while for exposure an illumination intensity of 2000 lx was selected. The reason was that the illumination intensity of a 8 volt head light at a distance of 1 meter was found to be approximately 2000 lx. The times of exposure were 10″, 30″, 1′ and 2′, and in order to know the subsequent visual performance for white light objects a Nagel's adaptometer was employed, while the illumination intensities of the test objects were represented by the logarithms of the adaptometer's values, into 7.9, 6.9, and 5.9. The illumination intensity of 7.9 turned out to be 0.15 lx when actually measured. The areas of the test objects were made into 5 grades of 1°, 3°, 5°, 7°, and 10°.

Results. (a) In the case of visual performance after light exposure, when the illumination intensity of test object was 7.9, no effects of environmental illumination intensity were received, but when the values were 6.9 and 5.9 and the area of the test object small, with an environmental illumination intensity of 200 lx, the visual performance became poor. (b) When the illumination intensity of exposure was kept fixed, then with prolongation of the time of exposure the visual performance became poor. But when the exposure time was above 1′ the changes were relatively small. (c) The lower the illumination intensity of the test object the poorer was the visual performance, and this tendency became greater the narrower the area of the test object became. (d) With increase in area of the test object the better became the visual performance for the object, while in the case of a value of 5.9, the effects due to differences in area of the test object became

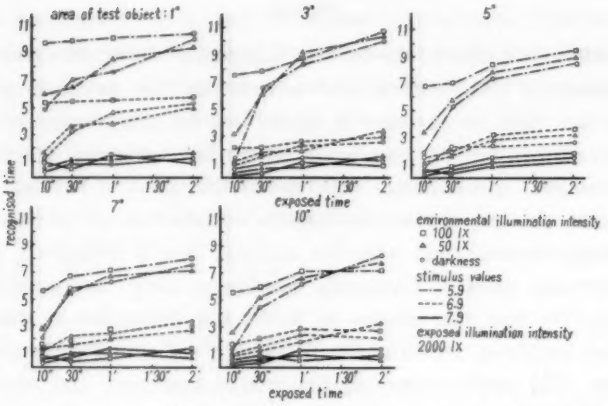

Fig. 1. The relationship between visual performance of white light test objects and environmental illumination intensity after exposure.

great but negligible in the case of 7.9. (Fig. 1. cf.)

2) Difference between monocular and binocular visual performance

As conditions, an environmental illumination intensity of darkness, and an illumination intensity of exposure equal to 2000 lx were employed. The times of exposure to light and the illumination intensities of the test object were the same as in experiment 1), while as area of the test object 5° was selected.

Results. When the times of exposure were 10″ and 30″, and difference in visual performance between monocular and binocular was not recognized because of the difference in illumination intensities of the test object. In the cases of 1′ and 2′, the times required for perception of the test object under the same conditions, are markedly shortened in binocular case. This tendency became more marked as the illumination intensity of the test object became lower.

3) Relation of visual performance after light exposure to employment of tinted wind shields

The environmental illumination intensity, illumination intensity of exposure and the illumination intensity of the test object were the same as in experiment 1). The area of the test object was 5°. In order to find the effects of tinted wind shields Matsuda's colored filters, IRO-1 and S-T 1, were used and experiments were conducted with monocular. Of these two filters IRO-1 was blue green and prevented the passage of infra red rays, while S-T 1 was light blue. The main wave length was 480 mμ. in case of IRO-1 and 400 mμ. in case of S-T 1. The conditions tested for comparative study were the following five, namely: (a) visual performance was measured after exposure to 2000 lx without the use of tinted wind shield, (b) the same as (a) but with the use of S-T 1, (c) when IRO-1 was used, and measurements made as in (b), (d) no tinted wind shield was used and the visual performance was tested after exposure to 400 lx. When S-T1 was employed

a 2000 lx exposure illumination intensity decreased to 400 lx and the exposure was conducted with this illumination intensity and the visual performance after exposure was measured. In this case the fall in visual performance after exposure when S-T 1 is used can be compared with that when no filter is used. (e) Exposed to 1200 lx and measurements mabe as in (d), (when IRO-1 was used an exposure of 2000 lx fell to 1200 lx; experiments were conducted with the same object as (d)).

Table 1. Environmental Illumination Intensity: 50 lx
Exposed time: 30″ Area of Test Object 50

	Stimulus value / exposed illumination intensity	recognised time		
		7.9	9.9	5.9
a	2000 lx	35″	2′5″	5′25″
b	S-T 1 2000 lx	31″	1′52″	4′58″
c	IRO-1 2000 lx	30″	2′2″	5′15″
d	400 lx	9″	37″	2′15″
e	1200 lx	13″	1″	3′1″

Results. As is indicated in table 1, when the experimental conditions (a), (b) and (c) were compared, it became clear that the wind shield selected did not lower the visual performance for the test object of white color. Such is believed to be due to the covering of the fall in visual performance for the test object by the decrease in illumination intensity during light exposure brought about by the tinted wind shield. When the results of (b) and (c) are compared with those of (d) and (e) the grade of deteration in visual performance as the result of wearing tinted glass becomes clear. (Fig. 2.cf.)

2. Visual performance for colored test objects

1) Effects of environmental illumination intensity on visual performance following exposure to light

The environmental illumination intensity and exposure illumination intensity were the same as in experiment 1, 1). Times of exposure were 10″, 30″ and 1′, and in order to measure the visual performance for colored light test objects after exposure a monochrometer was employed. As colored light test objects, monochromatic lights of red (670 mμ.), yellow (565 mμ.), green (510 mμ.) and blue (470 mμ.) were used, and these were standardized to be of equal brightness. After exposure there will first appear a negative after image and in the center of the test light will be perceived a sensation differing from the colored light before exposure, and this was termed light perception. The time for perception was recorded, followed by the time required for accurate perception of the colored light. The experiments were discontinued when it was believed that the negative after image had practically faded.

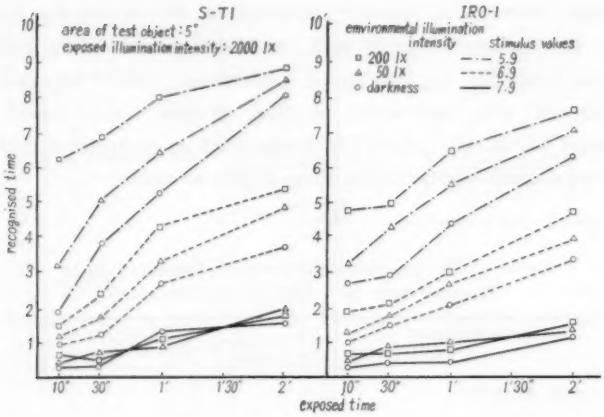

Fig. 2. Visual performance of white light test objects through colored glass after exposure.

Results. (a) The effects due to differences in environmental illumination intensity were maked with a light exposure of 10″, and in the case of an environmental illumination intensity of darkness, blue, green and yellow lights were perceived as different colored lights for some moments after exposure, namely blue light as purple, green as whitish, and yellow light as orange. When the environmental illumination intensity was 50 or 100 lx there are practically no cases where the colored light test object was perceived as a different color, but when the environmental illumination intensity was darkness, the illumination intensity of exposure 2000 lx and the time of exposure 10″, the after image remains for about 1′. In cases of 50 and 100 lx the time becomes shorter than under an environmental illumination intensity of darkness. (b) The exposure time influences the visual performance of the color after exposure, and the longer the time of exposure the longer will be the time required for accurate perception of the colored light, and similarly the after image persists longer. (c) The difference in visual performance resulting from exposure to different colored lights will be discussed from the point of the equal brightness of the colored lights. Consideration was also paid to the fact that the head light employed for exposure was a tungsten electric bulb of 2800°K. In all cases red was most easily perceived, but with blue, green and yellow errors easily occurred when the environmental illumination intensity was low. (Fig. 3. cf.)

2) Influence by use of tinted wind shields on visual performance for colored light objects after exposure

With environmental illumination intensities of darkness and 100 lx, exposure illumination intensity of 2000 lx, exposure time of 30″ and area of test object of 1°, the results were the same as in experiment 2, 1). The colored filters used were Matsuda's IRO-1 and S-T 1.

Results. (a) When IRO-1 and S-T 1 were used, the visual performance after exposure

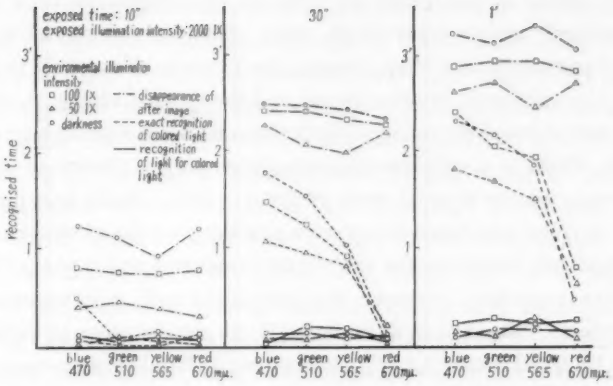

Fig. 3. The relationship between visual performance of colored light test objects and environmental illumination intensity after exposure.

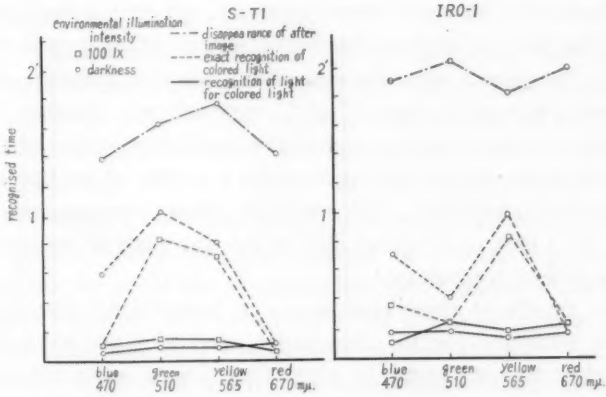

Fig. 4. Visual performance of colored light test objects through colored glass after exposure.

was good in the case of red light, despite of the use of these filters. Again when compared with the case where a colored filter was not used, the employment of IRO-1 was especially beneficial to visual performance for green and blue lights, but that for yellow light was generally bad. (b) When experiments were conducted with filters S-T 1 and IRO-1, and environmental illumination intensity of 100 lx, there was practically no glare during light exposure, and little after image after exposure. (Fig. 4. cf.)

Discussion

In order to assure road safety various improvements have to be made, and these are connected with the problems of image and background after image, tinted wind shields, differences in visual performances for white and colored lights, Doppler effect, age regu-

lation of drivers during night traffic, as well as impairments in color sense and light sense. In the present report experiments were conducted on powerful light exposure under various environmental conditions, namely the problem of exposure to head light, and apropos of this the problems of after image and tinted wind shields in relation to white and colored light test object. From the findings obtained some considerations will be made.

Regarding the effects of a previous light adaptation on the process of dark adaptation there have been many reports since the work of Treitel (1884). Again as regards the effects of different grades of light adaptation before dark adaptation on the increase in acuity of vision during dark adaptation, there are the reports of Yoshikawa and others (1937). However when the problem regarding exposure the head light and environmental illumination intensity is considered, attention has to be paid to the problem of visual performance for test objects being influenced by the environmental illumination intensity depending on the method of exposure. For example, the illumination intensity of a 8 volt head light placed at approximately 1 meter before the eyes is 2000 lx, and the times of exposure will vary greatly with the condition. At times this may be 2-3″, and in such a case the environmental illumination intensity before exposure will have a very great significance. But so far there has been no investigation conducted with attention paid to these matters. In view of this, in the present work and exposure illumination intensity of 2000 lx, and 4 grades of exposure time, namely from 10″ to 2′, were selected. Further, before exposure sufficient adaptation to environmental illumination intensities of darkness, 50 lx and 200 lx was made and measurements were made under a variety of conditions. As a special feature of the present experiment, and electronic counter was employed to measure the time accurately, in 1/1000 of a second, up to the perception of the test light under the various conditions following exposure.

Based on the results of these experiments it became clear that in order to assure road safety it is necessary for the illumination intensity of the test light to be above a limit of 7.9, namely that it should be more than 0.15 lx when measured immediately infront of the eyes, the reason being that test light objects with illumination intensities above this will be received easily with no influence by the environmental illumination intensity before exposure. Hence the illumination intensity of signals should be such that the test light when measured infront of the eyes is above 0.15 lx. When the test light is of low illumination intensity, the area of the test object small and the exposure time short, the influence of environmental illumination intensity will be received with ease. Especially when the test area is 1°, the visual performance after exposure becomes extremely poor. With a test area of 1° even when a physiological nystagmus is considered, sensation will be due to pure cones only. With limits of 6.9 and below it is believed that test light will be perceived mainly by the rods. Hence in case of short exposure, it seems that the senvironmental illumination intensity before exposure will influence greatly the sensitivity of the light receptor. From the above experimental results, when a small test light is seen in the dark after a short exposure to light, it would seem that it will be

better to keep the environmental illumination intensity previous to exposure, low. When the illumination of the test object surface was of limit 5.9, the time of light perception after exposure was long. From this it became clear that when a test object is gazed in the dark after exposure to strong light, it will require the long time of 11′ to perceive the test object when the luminosity of the test object is 0.0015 lx. This finding should receive attention by all road users, irrespective of the type of traffic under consideration.

Regarding the effects of accumulation of light stimulation due to both eyes the opinions of investigators have varied. Some have recognized the accumulative effects of light stimulation due to both eyes, while others have denied this. From the results of the present series of experiment, it became clear that during dark adaptation after exposure to 2000 lx for 10″ and 30″, there was practically no difference in the times of perception of light objects by one and both eyes, under the same conditions. But with exposures of 1′ or 2′ there was clearly noted a more rapid perception after exposure with both eyes. This finding deserves inclusion in the contraversy regarding the accumulation of light stimulation by both eyes, and indicates the necessity for normal light perception of both eyes in assuring road safety during night traffic.

When next we consider the influence of tinted wind shields, there is no literature in this country of experiments conducted on this. But lately, heat absorption glasses have come to be employed for wind shields of motor vehicles, and the colors are either blue or green. The writer believes that tinted wind shields lower visual performance of objects at night, but in the present work the relation between exposure to head light and visual performance after exposure was considered to have a great significance and experiments were therefore conducted along this line. Among the results a point that called for special attention was the difference in perception after exposure to 2000 lx when tinted wind shields were not used and when filters IRO-1 and S-T1 were used during and after exposure. Between them there was noted no marked difference, but at night when tinted wind shields were used visual performance became poor. However, exposed stimulation from head lights was definitely lowered and transmitted to the retina.

Colored wind shields with a balance of these two factors, should help in not only absorbing heat rays, but also lower the glare during exposure to head lights, while during daylight it prevents abnormally great light stimulation, and as such is of great value. From the results of experiments 1 (3), (b), (c), (d) and (e) where tinted wind shields were employed, it is clear how visual performance was lowered by the use of these appliances, and it is believed that this study will help in the selection of the proper tint of glass.

Next when we consider the perception for colored light object after light exposure it became clear that the effects of environmental illumination intensity are great when the exposure to light is strong and prolonged. In other words that the eye in the dark is influenced much more strongly than an eye at 50 or 100 lx when exposed to 2000 lx for 10″. In case of colored light objects the present experiments were conducted with an

object area of central fovea 1°, and as the sensation was due to pure cones, the function of the cones was excited by the environmental illumination intensity of 50 and 100 lx, so that even if an influence resulted from exposure to 2000 lx there probably be exhibited the ability to sense colored light constantly immediately after. On the other hand an eye in the dark shows exhibition of function of the rods alone and the cones are inhibited, so that when exposed to light of 2000 lx there will result marked excitation and the after image will be conspicuous. And as long as this after image remains the subsequent colored light objects will not be perceived accurately, and there will result perception which differs from the test object. In our work illumination was undertaken with a tungsten lamp of 300 watt 2800°K when the after image of a white light remained first as positive after image resulting from the sensation during light exposure. Later this changed into light green, followed by a negative image with the center red and the periphery pink. From the experimental results it was noted that after light exposure there was an instantaneous sensation of purple from blue light, of orange from yellow and a whitish yellow green from green. These are believed to be due to successive contrast resulting from the effects of positive and negative after images. In the case of exposure to head light against a background of relatively darnkess as in night traffic, it is clear that the visual performance of red colored test objects will not receive the effects of the after image easily, so that it must be admitted that the use of red light as a danger signal has been wisely selected. Further it was shown that when placed in an environmental illumination intensity of brightness such that a glare is not produced before exposure, the subsequent perception of colored light will be rapid and accurate. When the exposure time becomes long the more will be the time required for proper perception of colored light, so that caution should be paid not to gaze too long at a head light. The danger is great that tinted wind shields will cause false perception of colored light objects. The writer employing filters IRO-1 (light green blue) and S-T 1 (light blue), and found that S-T 1 facilitated the perception of blue light objects after exposure, and IRO-1 improved markedly the perception of blue and green light objects. From the above experimented results it is believed that the use of proper tinted wind shields will lessen the glare of drivers at night and remove the effects of infra red rays in daytime, and the use of such will not act as an obstacle.

Summary

In order to study the relationship of pereception of white and colored lights after exposure to such and the environmental illumination intensity the time of visual perception was measured with an electronic counter. Experiments were also conducted on the effects of tinted wind shields, and the following results were obtained.

1. In case of white light objects

i. From the results and the discussion, it was found that the area of stimulation after exposure should be above 3°, and the illumination intensity of the stimulated surface be greater than 0.15 lx. When the illumination intensity of the stimulated surface is low

and the time of exposure is short, it is better for the environmental illumination intensity to be low. However when the stimulating light after exposure is above 0.15 lx, the difference from the environmental illumination intensity does not produce a great influence on visual perception, so that it is preferable for the environmental illumination intensity to be bright in order to lessen the glare during the actual light exposure.

ii. Visual perception after prolonged exposure to light is better in case of binocular than in case of monocular.

iii. Tinted wind shields lessen more the glare than lower visual perception, and weaken the illumination intensity during light exposure. Hence, from the aspect of glare prevention during light exposure the use of a proper tinted glass is to be recommended.

2. In cases of colored light objects

i. The lower the environmental illumination intensity the longer is the time needed for disappearance of the after image becomes longer. When 4 monochromatic lights of the equal brightness were examined it was found that red light received least the effects of environmental illumination intensity.

ii. When tinted wind shields (filters IRO-1 and S-T1) were used, practically no after image was noted with an environmental illumination intensity of 100 lx, and the visual perception later was good. With these types of shields visual perception was relatively satisfactory for blue, green and red lights, while that for yellow was poor. Special attention should be paid to this when tinted wind shields of blue type are used.

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EXPERIMENTAL STUDIES ON CHRONIC RETROBULBAR NEURITIS

EXPERIMENTS ON TOXIC AND MENINGEAL NEURITIS WITH REFERENCE TO THE INFLUENCES OF VITAMIN A AND B_1 DEFICIENCY ON THE OPTIC NERVE

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Introduction

Etiologic classification of neuritis retrobulbaris presents many controversial problems. In Europe and America 80% of total cases of this disease are regarded as a symptom or an antecedent sign of multiple sclerosis, and according to Prof. Bing one person out of

1000 suffers from multiple sclerosis in Germany. On the other hand, this sclerosis has been regarded as an illness of very rare occurrence in Japan. Prof. Okinaka of Tokyo University reported that only 66 cases of multiple sclerosis, including suspected cases, had been recorded in Japanese medical literature up to 1955, but that all these cases were not pathologically typical. However, as for neuritis retrobulbaris, it is a common illness for Japanese ophthalmologists.

Are the Japanese racially more resistent to multiple sclerosis than the white people? Or is this difference in morbidity due to differences in food and other living conditions. These questions are unanswerable at present, as the essential nature of the disease is not yet sufficiently explored.

According to Biemond, Spilane reported that many German prisoners of war at El Alamein in North Africa fell victims to neuritis retrobulbaris on account of malnutrition, and De Ocampo and others reported the same thing about Japanese prisoners of war at various points of Asia, while Dekking stated that this ocular disease attacked many blond soldiers, but passed over their dark-haired comrades, though all of them were engaged in the same kind of labour with the same kind of diet. As long as real pathology of multiple sclerosis remains essentially unknown, it is impossible to decide definitely from these facts alone whether the nerve fibers of the optic, brain and spine of the coloured people offer more resistance to vitamin deficiency, toxicosis and various diseases than those of the white people; we are not yet in a position to say that Japanese people, even when they are exposed to a causative agent of multiple sclerosis, are affected very lightly, and develop only neuritis retrobulbaris, their cerebrospinal nerve fibers of the pyramidal tract and other parts incurring no demyelinative disintegration.

Since 70 years ago, a great many case reports on neuritis retrobulbaris have been published in Japanese journals of ophthalmology, but Miura's statement of 1911 that multiple sclerosis was an illness strange to Japanese people has never been questioned. Shimazono and others held beri-beri responsible for neuritis retrobulbaris, but Ichikawa objected to this theory, and on the grounds that nursing mothers were easy victims of this disease; that few patients with serious beri-beri complained of this ocular complication; and that more women were affected than men who were more liable to beri-beri, asserted that vitamin A deficiency was the real cause of this disease. Thus this problem on etiology of retrobulbar neuritis has long been a subject of hot dispute in the Japanese medical circles.

It is an age-long custom for the majority of Japanese city-dwellers to live on cleaned rice and take much low-fat diet, and so they are apt to suffer from vitamin A and B_1 deficiency. But it is not yet determined whether lack in vitamin A or B_1 really causes damages to the optic nerve. On the other hand, there are some ophthalmologists who assume, that toxic amblyopia which occurs under a variety of conditions usually begins with involvement of the retinal ganglion cells, which, then ascending, spreads to the optic nerve.

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Imachi noted the presense of arachnoiditis in retrobulbar neuritis; after he had conducted craniotomy on nearly 300 cases of serious chronic optic atrophy in a beginning stage, he came to the conclusion that circulatory disturbance of the intracranial fluid was an important factor of the optic nerve disturbance. In 1957 at the general meeting of the Japanese Society for Ophthalmology he published a report on his entrusted work on etiologic investigations into retrobulbar neuritis, and pointed out this fact.

Part of these investigations was assisted by his three coworkers, Kazikawa, Matsumoto and Ito. The former two made researches on toxic amblyopia combined with avitaminosis caused by vitamin A and B_1 deficiency and its relation with disturbance of the retina, optic nerve, arachnoid and brain; the latter on the influence of the intracranial fluid blockade caused by meningitis on the optic nerve function and nervous system. The present paper summarizes results of these researches.

Experiment I. On changes in the nerve fibers of the optic nerve, chiasm, and tract of the mouse in a state of vitamin A and B_1 deficiency.

Methods. Male mice with bodily weight of 20g. were divided into three groups. These three groups were respectively fed on normal, vitamin A and B_1 deficiency diets. The animals were decapitated every ten days, and serial sections were prepared. Observation of the optic nerve degeneration was pursued by means of Marchi staining. Below are shown composition of three kinds of diet, and fluctuations in the amounts of vitamin B_1 in blood and of vitamin A in liver.

Results. Pathologic Marchi's balls appeared in the optic nerve of vitamin A deficiency animals about 50 days after commencement of the experiment, and in that of vitamin B_1 deficiency animals about 40 days later, and then gradually increased in number. A long-term feeding with vitamin B_1 deficiency diet was interrupted because of high mortality, none of the animals fed on it being able to survive longer than 70 days. Deficiency of vitamin B_1 gave rise to a little earlier appearance of Marchi's balls than that of vitamin A, but this difference was not an important one.

Table I. Composition of Diets (One Day's Feed for a Mouse)

Diet / Ingredients	Normal Diet	V. B_1 Deficiency Diet	V. A Deficiency Diet
1. Cleaned Rice Flour	1.78	1.78	1.78
2. Purified Casein	0.9	0.9	0.9
3. Ebios	0.16	0.	0.16
4. Mixed Salts	0.12	0.12	0.12
5. Cod Liver Oil	0.06	0.06	0.
6. Water	Some	Some	Some
Amount of V. A	464 I.U.	464 I.U.	0
Amount of V. B_1	95.5 γ%	38.3 γ%	95.5 γ%

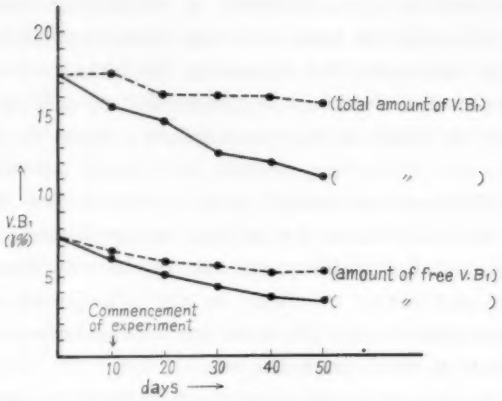

Fig. 1. Fluctuations in the amount of V. B_1 in blood
------ Rabbit fed on V.A deficiency diet
——— Rabbit fed on V.B. deficiency diet

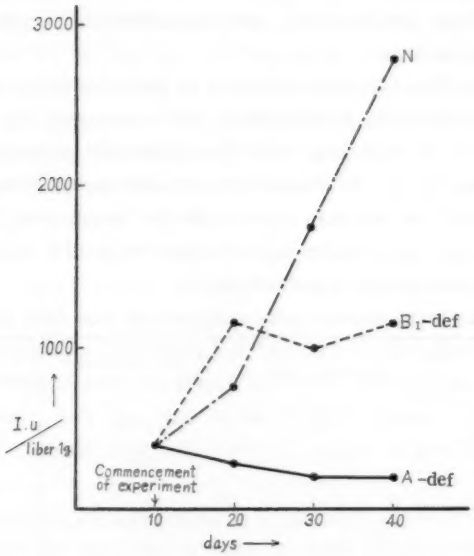

Fig. 2. Amount of V. A per 1 gr. of liver
—•—•— Rabbit fed on normal diet
------ Rabbit fed V.B_1 deficiency diet
——— Rabbit fed V.A deficiency diet

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Both with vitamin B_1 and A deficiency animals the central fibers of the optic nerve were involved to about the same degree as the peripheral. Involvement took place in the order mentioned in the prochiasmal portion, the optic chiasm, both pterygoid sides of the chiasm, transitional part from the chiasm to the tract, and the tract; and then extended up to the intracranial portion of the optic nerve and down to the intraorbital. After a time Marchi's degeneration intensified in these places, its degree in each place again following the order above mentioned. However, active multiplication of the ball was not noted in both intraorbital and intracranial portions. Generally-speaking, Marchi's balls were diffusely distributed, and no big solitary ball was findable, as in the case of Waller's degeneration. The balls were linked either in a threadlike or spindle-shaped form, and stained very dark. Compared with those of Waller's degeneration, these balls looked small. In short, long-term feeding on vitamin A or B_1 deficiency diet produces diffuse Marchi's degeneration which most often begins at the optic chiasm.

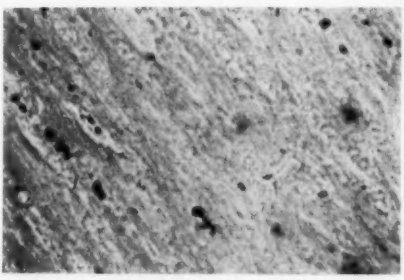

Fig. 3. Marchi's balls at the prechiasmal portion of the optic nerve. (70th day after feeding on V. A deficient diet. ×400.)

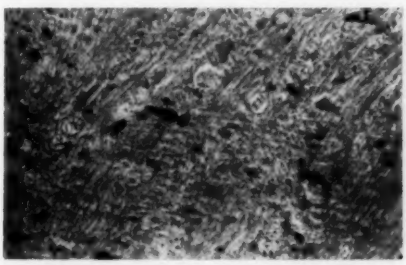

Fig. 4. Marchi's balls at the optic chiasma. (60th day after feeding on V. B_1 deficiency diet. ×400.)

Experiment II. Pathohistologic findings of the retina, optic nerve, and arachnoid of the mice respectively fed on normal, vitamin A,B_1 deficiency diets, and poisoned with acetic lead.

Methods. All the mice, whether they belonged to normal, vitamin A or B_1 deficiency diet group, were fed on normal diet for the first 10 days. For the next 10 days the two deficiency diet groups were fed on their respective deficiency diets. After this, all the mice were poisoned. Namely, the 2% solution of acetic lead were daily given orally to the experimental animals in 0.02 cc. quantities per 20 g.. The animals were then decapitated every ten days, and stained preparations were made from the retina, optic nerve, and arachnoid, using Marchi, Sugamo myelin sheath, hematoxylin-eosin double, Nissle and Glia stains. By the way, it was previously ascertained, that the amounts of vitamin B_1 in the blood, and of vitamin A in the liver would become insufficient in about ten days after commencement of deficiency diet feeding.

Results. Ten days later: Marchi's balls appear in small numbers in the optic chiasm and tract of the mice fed on vitamin A and B_1 deficiency diets. With some of vitamin B_1 deficiency mice, edema and small vacuoles are detectable in the nerve fiber layer of the

retina, while no retinal change is observable with normal and vitamin A deficiency diet mice. With both deficiency diet groups hemorrhages and collection of exudates are already present at the basis cerebri, especially at the cisterna chiasmatica, though proliferation of the arachnoid proper cells is still hardly observable.

Twenty days later: With both deficiency diet groups slight edema comes into the nerve fiber layer of the retina, and the ganglion cells also show slight swelling. Marchi's balls have multiplied to larger numbers at the optic chiasm and tract, and lesions at the subarachnoid space are more marked than ten days before. Some of the animals show destruction of the pial sheath of the optic nerve adjoining these lesions. Those of the normal diet group show no lesion in the retina and optic nerve as well as in the arachnoid at this stage of experiment.

Thirty days later: edema is observable in all the three groups of the nerve fiber layer of the retina, tigrolysis and swelling of the ganglion cells. But these changes are still partial and sporadic. No Marchi's balls are detectable in the optic nerve of the animals fed on normal diet. Proliferation of the arachnoid proper cells, and hemorrhages and exudative inflammation in the subarachnoid space are just as on the 20th day.

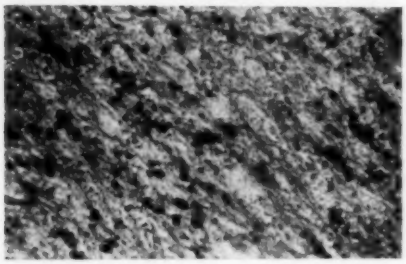

Fig. 5. Marchi's balls at the optic-tractus. (60 days per-oral administration of lead acetate. Normally-fed mouse. ×400.)

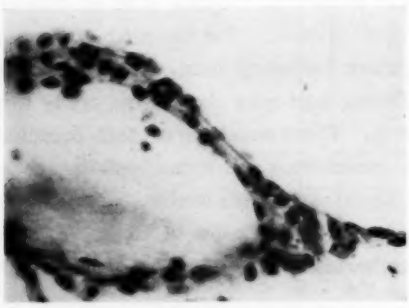

Fig. 7. Chromatolysis of the arachnoidproplia cell at the chiasma. (Poisoned with lead acetate 50 days on V. B_1 deficient mouse. ×400.)

Fig. 6. Exudates and hemorrhages in the chiasmal subarachnoid space. (20th day after lead poisened. V.A deficiency mouse. H.E. stain, ×100.)

Forty days later: With all the groups retinal changes are generally slight, about the same as on the 30th day. The first appearance in very small numbers of Marchi's balls occurs in the normal diet animals. With vitamin A and B_1 deficiency animals these balls show a gradual increase.

Multiplication of the arachnoid proper cells nearly ceases, exudataion and hemorrhages in the subarachnoid space are also now on the wane. But following

subsidence of these inflammatory changes, degeneration and adhesion of the proper cells begin. With the normal diet group the lesions are of course slight, but go through about the same pathologic changes as with the deficiency groups.

After the 40th day, retinal changes remain partial as ever, and in them no marked difference exists between the three diet groups. With all the animals Marchi's balls in the optic nerve gradually increase in number, the arachnoid presents the picture of degeneration and adhesion. The exudative process in the chiasmal subarachnoid space also gradually loses its activity.

Experiment III. The relation to vitamin A and B_1 deficiency of pathohistologic changes produced by aspidium extract poisoning in the retina, optic nerve and arachnoid of the mouse.

Methods. 220 male mice which had the average bodily weight of 20 g. were used. These animals were divided into three groups, and all the groups were previously fed on normal diet for 10 days. For the next ten days one group was fed on vitamin A deficiency diet, the other on vitamin B_1 deficiency diet, and a third one continned to be fed on normal diet. After this, all the animals were poisoned with daily oral administration of 0.005 g. of aspidium extract. The changes produced by poisoning in the retina, optic nerve, and arachnoid were pathohistologically investigated in the various stages of poisoning.

Results. Tigrolysis of the ganglion cells occurs first in the retina, followed by edema of the nerve fiber layer, and lesions in the inner granule and visual cell layers. These lesions become first observable 20-30 days later in normal diet animals, in vitamin B_1 deficiency ones 5 days later, and in vitamin A deficiency ones 5-10 days later. Having no special place of predilection, these lesions first appear sporadically in the retina, and after a while become diffuse. Hematoxylin-eosin, axis cylinder, glia and myelin sheath stainings discover lesions in the optic nerve of the normally-fed 30 days later, and in that of deficiency diet mice 5 days later.

The primary sites of involvement are usually the optic chiasm and the latter half of the intracranial portion. Gradually diffuse spreading of the lesion takes place, but the chiasm, intracranial portion, and tract are nearly always more seriously involved than the intraorbital portion.

The degree of involvement is highest with the vitamin A deficiency diet group. Vitamin B_1 deficiency mice, too, show a stronger and earlier involvement than the normally-fed.

As to the findings in the chiasmal arachnoid, subarachnoid space and dural membrane, exudates collected in the subarchnoid space of the normally-fed 10-20 days later, and after 40 days the arachnoid endothelia showed degeneration and adhesion to the dural membrane, while with vitamin B_1 deficiency group exudates collected in the subarachnoid space already two days after poisoning, and about 15 days later the endothelia were developing degeneration and adhesion. As to the vitamin A deficiency group, exudation began to collect in the space 2-5 days later, and after 20 days degeneration of the

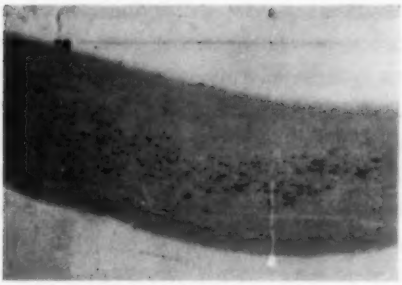

Fig. 8. Marchi's balls of the optic nerve. (80 days poisoning with Aspidium extract on mouse. normally-fed. ×100.)

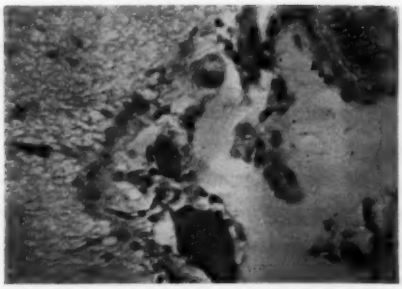

Fig. 9. Exudates in the prechiasmal subarachnoid space. 50 days poisoning with Aspidium extract on V.A deficient mouse. ×400.)

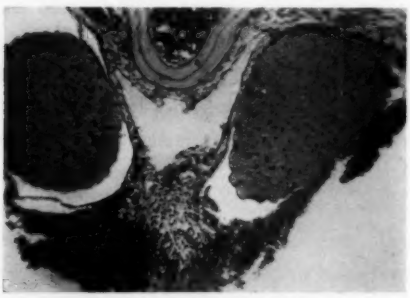

Fig. 10. Exudates and hemorrhages in the chiasmal subarachnoid space. (30 days poisoning with Aspidium extract on V. B_1 deficient mouse.)

endothelia set in. The adhesion was demonstrable from 10 days later.

In all the groups, no thickening of the arachnoid, cellular infiltration, pathologic changes of the blood vessels, and thickening of the dural membrane were demonstrable. The arachnoid about the chiasm was most seriously damaged. In short, poisoning took effect in the normally-fed later than in vitamin A and B_1 deficiency diet animals.

Experiment IV. Experimental studies on arachnoiditis and optic nerve disturbance of rabbits inoculated with the combined vaccine against typhoid fever and paratyphus.

Methods. As the injection of vaccines against typhoid fever and paratyphus, though capable of producing retrobulbar neuritis, rarely does so, and as meningeal neuritis is one form of retrobulbar neuritis, the combined vaccine against typhoid fever and paratyphus was directly injected into the cisterna magna in this experiment. Male albino rabbits with bodily weight of about 2kg. were used as experimental animals. They were divided into three diet groups. A normal diet consisted of 300 g. bean curd refuse (Okara) and vegetables; and a vitamin A deficiency diet of 300 g. bean curd refuse from which fat had been removed with ether, and which, after being dried, had been brought back to normal humidity, and 0.5 g. of a multi-vitamin preparation, Panvitan, from which vitamin A had been removed. As for a vitamin B_1 deficiency diet, it was prepared with 300 g. bean curd refuse, the vitamin by heating B_1 of which had been destroyed at 120°C for 30 minutes under 2 atmospheric pressures. (The amount of these diets is for a rabbit a day.)

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Normally-fed rabbits were put to experimental use after two-week feeding, vitamin A deficiency ones after 90-day feeding, and vitamin B_1 deficiency ones after one-week feeding. Before inoculation of the vaccine into the cisterna magna, it was clearly ascertained that the amounts of vitamin B_1 in blood and of vitamin A in liver had sufficiently decreased in rabbits fed on their respective deficiency diets.

The 1 : 10 diluted solution of the vaccine was inoculated in 0.5 cc. quantities into the subarachnoid space in the occipital cisterna magna. Experimental animals in each diet group were inoculated either once or twice. The second inoculation in which the same amount of the inoculum was used as in the first, took place one, two or three weeks after the first inoculation. At various intervals, the 14th day after the last inoculation the experimental animals were killed and subjected to investigation. Those which presented very striking arachnoid lesions were subjected to a long-term observation.

Results. (1) With normally-fed rabbits inoculated once with the vaccine the arachnoid surrounding the cisterna chiasmatica was found to be in a light inflammatory condition, but no endothelial proliferation was demonstrated. Three weeks after inoculation Marchi's balls appeared in very small numbers.

(2) With normally-fed rabbits reinoculated 7 or 14 days later arachnoiditis was gain none too serious, and arachnoid proliferation and thickening, too, were almost negligible, as in the cases of those inoculated once. However, those reinoculated three weeks later showed exudative inflammation of the arachnoid accompanied by extremely severe hemorrhages. This inflammation was followed by degeneration and active proliferation and thickening of the chiasmal arachnoid. Circulation of the intracranial fluid was also disturbed by adhesion of the arachnoid to the pia mater. Thus reinoculation at an interval of 21 days gave rise to strong allergic inflammation, and produced marked lesions in the arachnoid.

It was found on investigation on the 14th day after reinoculation that reinoculation, whether it was done one, two or three weeks after the first inoculation, had caused almost the same slight damages to the myelin sheath of the optic nerve. Namely,

Fig. 11. Marchi's balls of the optic nerve, normally-fed rabbit. (3 weeks after the inoculation of combined vaccine. ×100.)

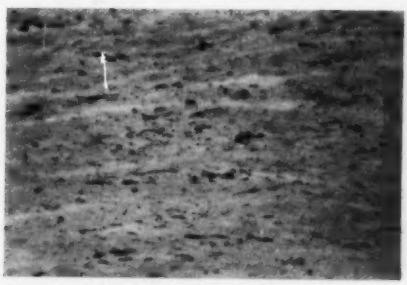

Fig. 12. Marchi's balls of the optic nerve, normally fed rabbit. (2 weeks after the reinoculation with 3 weeks interval. ×100.)

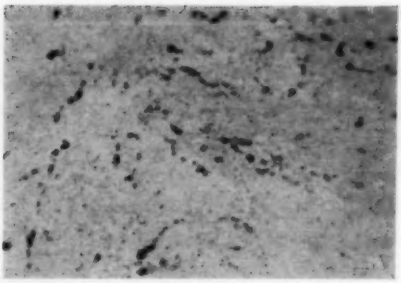

Fig. 13. Marchi's balls in the optc nerve, V. B_1 deficiency rabbit. (2 weeks after the reinoculation of the combined vaccine with 3 weeks interval.)

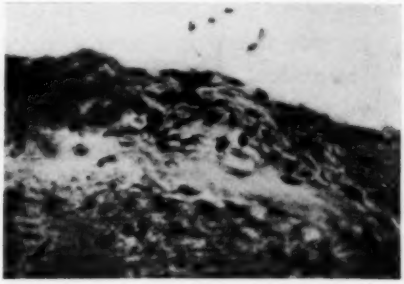

Fig. 14. Thickening and proliferation of the chiasmal arachnoid. (2 weeks after the reinoculation in normally fed rabbit.)

though the arachnoid was liable to strong allergic inflammation, the optic myelin sheath proved itself very resistant.

(3) Those rabbits which were reinoculated 7 or 14 days later, and showed slight arachnoid lesions were also found on a long-term observation hardly to suffer from arachnoid and Marchi's optic nerve degeneration. On the contrary, with those which were reinoculated 21 days later, and presented serious arachnoid lesions, the arachnoid adhered to the pia mater, and disturbed circulation of the intracranial fluid; and by means of Marchi's staining destruction of the myelin sheath was constantly demonstrated. Very extensive demyelization of the optic nerve was noted with a rabbit decapitated 90 days later.

Though the same amount of the vaccine was inoculated, those animals which developed severe arachnoid lesions, and accordingly had the intracranial fluid circulation blocked suffered from heavier damages to the optic nerve.

(4) With vitamin B_1 deficiency rabbits arachnoid proliferation, thickening and adhesion to the pia mater were not so serious as with the normally-fed, but Marchi's degeneration was more advanced. This was because deficiency of vitamin B_1 lessened allergic inflammation, namely, antibody-producing power of the animal, but on the other hand, this avitaminosis was responsible for aggravation of the optic nerve disturbance.

(5) Vitamin A deficiency rabbits which received reinoculation suffered from about the same arachnoid proliferation and thickening as the normally-fed, those reinoculated 21 days later presenting the severest lesions. But with them the optic nerve was by far more seriously affected than with the normally-fed; and it was proved that deficiency of vitamin A was also one of the factors responsible for disturbance of the optic nerve in rabbits.

Conclusions

(1) Either deficiency of vitamin A or that of vitamin B_1 alone is capable of producing

Marchi's balls in the optic nerve. Thus caused primary degeneration of the murine optic nerve was recognized in the present experiment.

(2) Continued oral administration of acetic lead causes lesions in the retina, optic nerve, and arachnoid of not only vitamin A and B_1 deficiency mice, but the normally-fed as well. But thus caused lesions are more severe on the part of vitamin A and B_1 deficiency animals. It is unthinkable, that lesions always spread from the retina upward to the optic nerve; in the present experiment the retina and the optic nerve were independently and separately damaged.

It is highly probable that lesions in the arachnosd membrane and subarachnoid space surrounding the optic nerve have much to do with latter's degeneration.

(3) Poisoning with aspidium extact also causes lesions in the retina, optic nerve, and arachnoid of the mouse. With the normally-fed, the arachnoid is first involved, and then the retina and the optic nerve. However, with vitamin A and B_1 deficiency mice the retina and the optic nerve are separately and non-continuously involved to fall into degeneration. In this case, too, it cannot be said that the retinal involvement spreads upward to the optic nerve.

(4) In case of poisoning with aspidium extract, too, arachnoiditis, especially exudative inflammation in the subarachnoid space, and endothelial degeneration always occur, and are severest in the chiasmal region where the optic nerve begins to degenerate, and presents the most serious aspect of degeneration.

(5) Vitamin A or B_1 deficiency seriously aggravates pathohistologic changes of the arachnoid, optic nerve, and retina brought about by poisoning.

(6) The diluted solution of the combined vaccine against typhoid fever and paratyphus, if inoculated once into intracranial space of the rabbit, causes a very mild inflammation, but if twice at an interval of three weeks, it is able to produce a very violent allergic inflammation, namely, arachnoid endothelia at the cisterna chiasmatica become proliferative, thickened, and then adhesive to the pia mater, thereby causing circulatory disturbance of the intracranial fluid. These changes are followed by Marchi's degeneration and demyelization of the optic myelin sheath. If the vaccine is inoculated twice in the same quantities at a shorter interval, a violent allergic inflammation does not occur. In such a case arachnoid adhesion and thickening are also slight, and damages to the optic nerve are almost negligible. From this fact it may safely be inferred that circulatory disturbance of the intracranial fluid exerts very injurious influences on the function and nutrition of the optic nerve.

(7) The animals fed on vitamin A deficiency diet, when reinoculated 21 days later, develop allergic arachnoiditis of about the same degree as the normally-fed, and between the two there is also little difference in arachnoid thickening and proliferation. But in spite of this the former incur by far a more serious degeneration of the optic nerve.

(8) The animals fed on vitamin B_1 deficiency diet, when reinoculated, develop arachnoiditis of lighter nature than those fed on normal and vitamin A deficiency diets. But

their optic degeneration is most severe.

(9) Vitamin A or B_1 deficiency markedly hastens disintegration of the optic myelin sheath in meningeal neuritis.

(10) It may be concluded from the above experimental facts that deficiency of vitamin B_1 or A also clinically plays an important role in preparing a predisposition to the optic nerve disease.

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STUDIES ON THE CENTRAL RETINAL ARTERIAL PRESSURE

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Much has been written about studies on the central retinal arterial pressure (CAP). However, mosts studies not gone more over than to show that values of CAP corresponding with ophthalmoscopic pictures in any each group have large variation. Therefore, it is not worth while to estimate values of CAP so far as its systolic or/and distolic pressure is concerned, and these values provide little useful addtion to our knowledge about the status of the individual. It is necessary for clinicical significance of CAP to analyse and manage its values mathematically.

Only several papers on the management of CAP values have been published by Weigelin, Müller and Peter.

The author attempted to find the method of mathematical analysis and management of CAP values and these results were compared with those by Weigelin's method and were applied to Keith-Wagener's classification in hypertension.

The investigation of these results shows that hypertensive diseases are able to be more correctly classified with regard to diagnosis and prognosis by the management of CAP values.

Method and Materials of Investigation

Values of CAP and brachial blood pressure (BrP) were carefully measured by Bailliart's ophthalmodynamometer and Riva-Rocci's Sphygmomanometer respectively. Dynamometric readings in grams were converted into millimeters of mercury from the Hasebe's graph.

Author's formula conducted by the management of CAP values is as follows:

$$\frac{\text{R.Syst}-\text{R.Diast.}}{\text{R. Diast.}} + \frac{\text{Br.Syst}-\text{Br.Diast.}}{\text{Br. Diast.}}$$

R.……CAP, Syst.……Systolic pressure

Br.……BrP, Diast.……Diastolic pressure

This formula is designated as comparative different pressure ration (comp. diff. p. ratio).

The materials consists of 40 normal persons aged from 20 to 59 and 149 hypertonics aged from 35 to 78.

Results

The investigations was carried out according to the criteria of the Keith-Wagener's classification in hypertension. In general, brachial systolic and diastolic pressure values, as in Table 1 and 2, inclined to increase with the progress of group in Keith-Wagener's classification. And retinal systolic and diastolic pressure values, as in Table 3 and 4, also revealed the same tendency.

Table 1. Relation of Br. Syst. to Grouping

K-W	I	II	III	IV
lowest	142	144	170	200
highest	200	250	240	250
mean	164.3	182.1	206.6	221.7

Table 2. Relation of Br. Diast. to Grouping

K-W	I	II	III	IV
lowest	72	74	90	110
highest	110	120	134	150
mean	91	97.5	114	138.5

Table 3. Relation of R. Syst. to Grouping

K-W	I	II	III	IV
lowest	72	72	91	106
highest	117	134	124	138
mean	92.3	102	109.7	118

Table 4. Relation of R. Diast. to Grouping

K-W	I	II	III	IV
lowest	32	34	50	62
highest	58	82	70	92
mean	48.6	55.3	61.3	73

Table 5. Relation of $\frac{\text{R. Syst.}}{\text{Br. Syst.}}$ to Grouping

K-W	I	II	III	IV
lowest	0.42	0.42	0.48	0.51
highest	0.72	0.72	0.60	0.55
mean	0.563	0.566	0.533	0.531

Table 6. Relation of $\frac{\text{R. Diast.}}{\text{Br. Diast.}}$ to Grouping

K-W	I	II	III	IV
lowest	0.38	0.40	0.48	0.51
highest	0.76	0.82	0.74	0.64
mean	0.538	0.570	0.541	0.557

Table 7. Relation of $^{rs}/_{rd}$ -Carot to Grouping

K-W	I	II	III	IV
lowest	0.92	0.82	0.80	0.90
highest	1.36	1.28	1.14	1.06
mean	1.091	1.044	1.029	0.998

Table 8. Relation of Comp. D.P. Ratio to Grouping

K-W	I	II	III	IV
lowest	0.60	0.55	0.52	0.64
highest	2.12	2.35	1.26	1.04
mean	1.174	1.017	0.983	0.881

Table 9. Relation of D.P. Ratio to Grouping

K-W	I	II	III	IV
lowest	0.31	0.36	0.38	0.42
highest	0.97	1.12	0.68	0.55
mean	0.62	0.57	0.53	0.50

The ration of retinal blood pressure to brachial one, as in Table 5 and 6, was about 0.5 in group III and IV, while in group I and II, this ratio was more than 0.6.

The average values of Weigelin & Peter's different pressure ratio (diff. p. ratio) $\left(\frac{\text{R.Syst}-\text{R.Diast.}}{\text{Br.Syst}-\text{Br.Diast.}}\right)$ revealed the tendency to decrease with the progress of groups and as rule, in group III and IV, this ratio was merkedly small.

Carotis volume elasticity coefficient of Weigelin & Müller = rs/rd-Carot. has also tendency to decrease with the development of the groups, as shown by Table 8, and particularly in

group III and IV was very small.

Finally, it was found that mean values of Sasamoto's comp diff. p. ratio also incleined to decrease progressively with the groups, as shown by Table 9.

Discussion

It is demonstrated by Wezler & Böger that the important factors which maintain blood pressure are output per beat, peripheral resistance and resistance of blood vessels. Therefore, changes in some of these factors may bring alterations in blood pressure. Since it is suggested that hypertension are usually associated with the involvements of heart, it is considered to have attentively to evaluate CAP.

Also in my investigations, CAP value showed the tendency to increase with the development of fundus pictures, as had been reported in many literatures, but since the same correlation was found in brachial blood pressure, there is no important practical value of clinical use of ophthalmodynamometry necessary for more difficult technique.

Furthermore, in investigations of retinal-brachial blood pressure ratio, if CAP may increase parallel with brachial blood pressure, this ratio provide also no clinical significance, it seems to be clinically valuable, although it has not yet been completely investigated.

After all, it is considered that value of CAP by itself is of little worth in clinical aspects, then the author has attempted to analyse and manage value of CAP to make it clinically helpful.

The author has found, being based on Wezler & Bögers or Weigelin's views, that there are parallel correlation between BrP and CAP and close relationship between Syst−Diast. and Diast., that is, $\frac{\text{R.Syst}-\text{R. Diast.}}{\text{R. Diast.}}$ and $\frac{\text{B.Syst}-\text{Br. Diast.}}{\text{Br. Diast.}}$ (designated as comparative different pressure).

Furthermore, the author introduced a new formula previously mentioned.

$\frac{\text{R.Syst}-\text{R. Diast.}}{\text{R. Diast.}}+\frac{\text{Br.Syst}-\text{Br. Diast.}}{\text{Br. Diast.}}$ which is designed as "comp. diff. p. ratio" and may be closely related to the resistance in brain blood vessels.

In this investigation, it has been revealed that comp. diff. p. ratio is to be related intimately with diff. p. ratio (Weiglin & Peter) rs/rd-Carot. (Weigelin & Müller).

Consequently, it is considered conclusively that comp. diff. p. ratio clearly reveals the resistance in brain blood vessels, that is the resistance in brain blood vessels increases with decrease of comp. diff. p. ratio.

Average value of comp. diff. p. ratio in normal persons was calculated to be 1.364 and the value which is considered to be related to the increased resistance in brain blood vessels was below 1.10.

Furthermore, in the investigation on the relationship between retinal-brachial diastolic pressure ratio and comp. diff. p. ratio, both were found to be closely correlated each other, that is, retinal-brachial ratio is the greater, comp. diff. p. ratio is the less, and

then these results are now considered to support Bailliart's opinion that increased retinal-brachial pressure ratio may show the presence of a greater resistance in brain blood vessels and Espildora's suggestion that increased retinal-brachial pressure ratio may reveal "brain hypertension".

The foregoing investigationg into the behaviour of diff. p. ratio, rs/rd-Carot. and Sasamoto's comp. diff. p. ratio in Keith-Wagener's classification revealed that all these values become more low respectively with development of the fundus pictures from group I to IV. namely, as the fundus pictures become aggravated, the resistance in brain blood vessels has the tendency to be more increased.

Most of these results coincide with those of a few other workers, apart from those on retinal-brachial pressure ratio, that is, retinal-brachial pressure ratio can not always reveal the degree of resistance in brain blood vessels, and then it is the purpose in this pressent investigations to find the best method for analysis and management of blood pressure values, by which the degree of resistance in brain blood vessels may be more clearly and exactly revealed, and the author's comp. diff. p. ratio, has been found to be more useful formula for the purpose.

The prognostic studies on hypertensive patients showed that when cerebral hemorrhage may occure in group II, value of comp. diff. p. ratio has been always markedly low, although pressure value has not been always high.

Most of patients in group IV died from lesions of kidney or brain and these cases had have very low value of comp. diff. p. ratio. It is most interest and significant that low value of comp. diff. p. ratio has revealed the increased resistance in brain blood vessels in these patients. With a view to clinical application of comp. diff. p. ratio, the author further investigated the correlation between comp. d. p. ratio and retinal-brachial pressure ratio in relation to Keith-Wagener's classification.

When the patients are divided into two groups, namely showing over 0.61 and below 0.60 in value of retinal-brachial preesure ratio, the group showing below 0.60 may belong to every group in Keith-Wagener classification, while most of the patients in the group

Fig. 1.

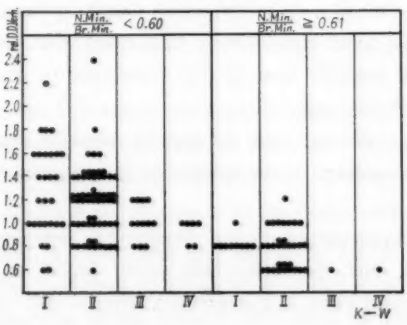


Fig. 2.

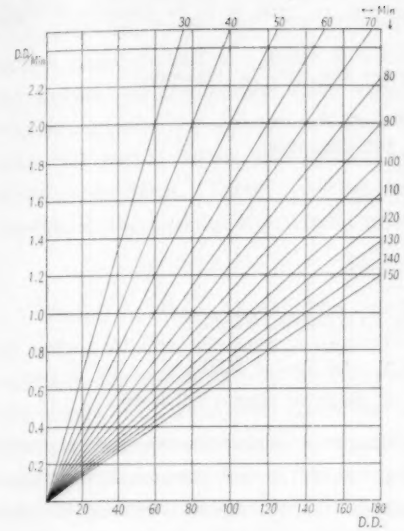
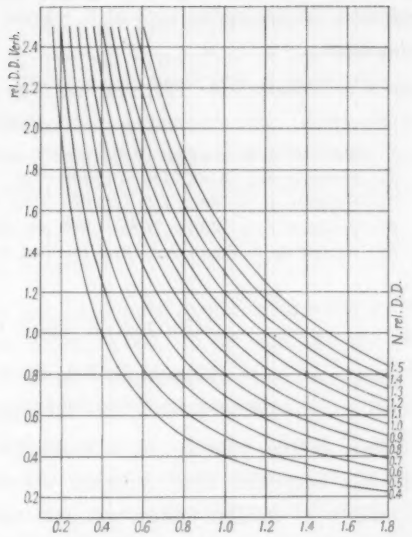


Fig. 3.



showing over 0.61 reveal the fundus picture belonging to only group I and II, moreover, in both groups, the fundus appearances become more aggravated with diminution of values in comp. diff. p. ratio.

Thus, the author believes that comp. diff. p. ratio is of great value for the clinical use with regard to diagnosis and prognosis in hypertensive diseases.

Finally, the author devised the nomogram for calculation of comp. diff. p. ratio by which its value is to be simply and readily calculated (Figs. 2 and 3).

First, either value of $\frac{\text{Syst-Diast.}}{\text{Diast.}}$ in CAP and BrP is respectively calculated by use of fig. 2. and then value of comp. d. p. ratio is to get by use of fig. 3.

Summary

1) It has been found that both brachial and retinal blood pressure, as a rule, tend to be raised with aggravation of fundus pictures.

2) Comp. d. p. ratio devised by the author is closely related with diff. p. ratio and rs/rd—Carot., that is, these values have a tendency to decrease with the development of fundus pictures.

3) The patients in Keith-Wagener's group II, in which com. diff. p. ratio is diminished, have an inclination to occurrence of cerebral hemorrhage and the author's view that general prognosis is poor in those which fall into group IV, has coincided with that of other previous workers.

4) It is considered conclusively that comp. diff. p. ratio is very useful to classify more

definitely hypertensive diseases in Keith-Wagener's classification, and then the author has presented the nomogram by which values of com. diff. p. ratio is to be easily and readily calculated.

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IMPEDANCE TEST OF THE CORNEAL TISSUE

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Just as the values that indicate the conductance or dielectric constant of various materials represent their differing characteristics, which is our common knowledge, an impedance of cellular tissues will lead us to conclude that the figures obtained therefrom have a direct connection with their organic characteristics, especially the ion permeability. This fact has already been reported by Holt, Cogan and Friedenwald.

For a conductivity test, a new method by means of the high frequency has recently been introduced. This has been proved to furnish us with figures representing plate or grid-currents, or voltages, or frequencies of the resonance circuit, with which we may express the conductivity of the test materials. Taking advantage of this new method, we conducted our experiments upon vitreous body, aqueous humor, serum, lens, retina and iris, each in milky liquid form. Due to difficulties attendant upon mutual induction and instability of the figures, we have not been able to experiment upon the tissue itself.

It is considered highly probable that the low frequency and the high frequency may have different meanings in the tissue.

In this report, we intend to mention the results of our observation upon various changes of electrical resistance found in the tissues when tested by means of an impedance bridge, approximately the same device as was used by Hold and Cogan. In addition, we will go a step further by taking up the problems connected with the phase angle in the following paragraphs.

Experimental Methods

An impedance bridge was used for finding the resistance and the capacitance, two important factors concerning the tissue impedance. Parallel circuits of the resistance and the capacitance were adopted as equivalent circuits of the tissue and were inserted into the bridge. The voltage of the audio oscillator, the bridge source, was adjusted at 0.75 V by a valve volt-meter. A cathod ray oscillograph, a valve volt-meter and headphones

were used for null indication. Platinized platinum was used as electrodes and salt solution of 0.9% concentration of Ringer's solution was poured into the conductivity cell thus connecting the tissue and the electrodes. The cornea for the experiment was extracted from a fresh eye-ball of a cattle or a rabbit. The eye-ball was cut off at the sclera near the limbus and the cornea after separation from the eye-ball was steeped in Ringer's solution for about ten minutes and then was tested in the conductivity cell. After determining the normal value, comparative studies were conducted among several cases under various conditions. Direct reading of the resistance and the capacitance was made possible on the basis of the already known value in the bridge.

Experimental Results

Changes in Phase Angle. The result of the test conducted upon the cornea of a rabbit by means of five different frequencies (3,500; 5,500; 8,000; 13,000 cps) of the audio oscillator, the bridge source, are recorded in the table. The resistance and the capacitance will readily be obtained from the bridge indication. On the basis of formula, $Xc=(2\pi fc)^{-1}$, the capacitive reactance can be computed from capacitance and frequency. From Xc and the resistance, the so-called real resistance (wahrer Widerstand) which means the resistance when frequency is infinite, can be computed by means of extrapolation. This is expressed in R_∞. In this way, the part of the resistance that changes in accordance with frequency can be obtained on the basis of formula $\Delta R=R-R_\infty$. Our computation of $2\pi fc\Delta R$ produces a constant value and shows that it has no connection with frequency. This constant value represents the phase angle of corneal impedance, which testifies that the theory of uniformity of phase angle is also applicable in the case of the cornea. As seem in figure 1 (a), the phase angle of the rabbit cornea was found to range from 18° to 26° with the average standing at 22°, which can be regarded as conspicuously small compared with

Table 1. The Sample Value obtained to find the Phase Angle on the Basis of Computation

Frequency (cps)	Capacitance (μf)	Capacitive Reactance (Ω)	Resistance (Ω)	ΔR (Ω)	Phase Angle (tan φ)	φ (°)
3500	0.0460	990	618	361	0.365	20.1
5500	0.0390	741	547	290	0.392	21.4
8000	0.0333	599	482	225	0.376	20.6
13000	0.0284	431	418	161	0.374	20.5
∞			(257)			

Frequency: This concerns the oscillator used as bridge source. Capacitance and Resistance: Directly obtainable from the known capacitance and resistance inserted in the bridge-arm. Capacitive Reactance: Obtainable from computation of fomula $Xc=(2\pi fc)^{-1}$ ΔR: Obtainable on the basis of $\Delta R=R-R_\infty$. R_∞ means the resistance when frequency is infinite and can be obtained from resistance and Xc by extrapolation. Phase Angle: To be computed from tan $\varphi=2\pi fc\Delta R$. φ: Obtainable from trigonometrical table.

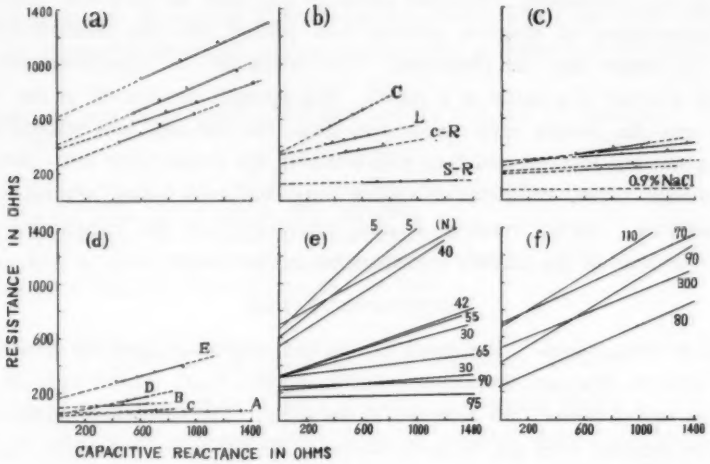

Fig. 1. Changes of the phase angle witnessed under different conditions. (a): Four instances obtained from a normal rabbit cornea. (b): Comparative studies between C (central part of the cornea), L (encircling part), C~R (Conjunctiva~retina), S~R (sclera~retina). (c): Changes witnessed in formalin fixed cornea. (d): Changes caused when the epithelium were scratched off to different extents. (The scratches become milder in the alphabetical order, (A) showing the deepest.) (e): Changes brought under the influence (5 min to 90 min) of guanidine hydrochloride (0.5 M). (f): Changes under cyanic acid (M/1000) influence.

that of the skin (62 to 77), the nerve (64) and the muscle (66) and even does not come up to the 45° shown by the semipermeable membrane. This is a solid evidence to the fact that the polarizations capacity induced on the cornea is considerably leaked by ion permeation and that the ion permeability in the cell membrane of the cornea is comparatively high.

Whereas the foregoing test results concern the central part of the cornea, we further compared them with those of the encircling part, upon which it was found that the phase angle was smaller in the latter and came up to somewhere between 27% and 97% of the former and showed a considerable variability. The average value obtained out of nine instances stood at 57% of that of the central part. In the conjunctival section that covers from the conjunctiva to the retina, the average out of four instances was 6°. This is a still smaller figure and with extraction of the conjunctiva, it further dropped to 3° that can even be considered as a full permeable point.

From these facts witnessed in our test, we can reach the conclusion that the most difficult section for ion to permeate is the center of the cornea and it is easier in the part that encircles it and it is still easier in the conjunctiva section, and in the part from below the conjunctiva to the inside of the eye-ball there exists nearly a full permeable condition. This is as shown in figure 1 (b).

An experiment was conducted upon the cornea that had been preserved in formalin of 10% density for several consecutive days and we came to know that the resistance had tremendously dropped even to the point of R_∞. As frequency becomes infinite, the cell membrance entirely loses its resistance, the fact that the resistance was found to be close to R_∞ indicates that ion permeability rose to a conspicuously high point due to destruction of the cell membrane under the fixative influence of formaline. Decrease of the phase angle on the other hand was noted as another symptom. In other words, whereas the normal cornea had an average of 22°, it markedly dropped and ranged from 2° to 8° averaging 5.2° in the case of the formalin fixed cornea, which was as small as if the experiment had been conducted without the cornea in the salt solution of 0.9% density connecting the electrodes. This means the formalin fixed cornea hardly resists ion permeation, several instances of which are shown in figure 1 (c).

Figure 1 (d) show the result of an experiment conducted upon the cornea the epithelium of which deliberately scratched off. The extent of the scratch becomes milder in the alphabetical order. Results shows that the phase angle dropped in accordance with the extent of the scratch and it even came down to zero in the case of example (A). A mild scratch was given to the cornea surface in the case of (E), yet nevertheless it showed a sharp drop even to 50% below normal. This tells up how much influence is brought upon the permeability of the cornea by scratching the epithelium.

Figure 1 (e) shows the action upon the cornea when guanidine hydrochloride (0.5M), a protein-denaturating agent, was used. After five-minute experiment, the phase angle showed a tendency to rise above the normal figure. Up to sixty minutes, it dropped below normal, but it did not necessarily show precise relation with the length of time. As the experiment lasted for 90 to 95 minutes, the phase angle plummeted close to zero, which proves that the cornea permeability is also remarkably influenced by the degeneranion of organic protein.

When the inhibition of metabolism by cyanic acid (M/1000) was examined, a 300 minute test produced no specific change as is shown in figure 1 (f).

Changes in Electrical Resistance. It is common knowledge in physical chemistry that practically the same theory can be applied to the electric resistance in electrolytic solution as in metalic conductor where we compute the resistance in ohm. Holt and Cogan brought this idea into their research on the cornea permeability. It can safely be presumed that the conductance, which is the reciprocal of the resistance, is in fact directly related to the ion permeability of the cornea. In the following paragraph, we would like to mention what variations were brought about in the cornea electrical resistance by different chemical and physical stimulations. Average figures were adopted for the current report as shown in figures 2 and 3. The variations of the resistance, both upward and downward, were determined percentage-wise against the normal value. Upon comparing the acid and the alkali actions upon the resistance, it was readily discovered that the letter had worked a far stronger action than the former. For instance, when caustic soda of more than 2.5%

← per cent. →
50 (−) 0 (+) 50
1.2% NaOH (5 sec.)
12.0% H2SO4 (5 sec.)
5% ZnSO4 (10 min.)
0.05% AgNO3 (10 min.)
5.0% Protargolum (10 min.)
5% Cocain (15 min.)
3% Cornecain (15 min.)
4% Xylocain (15 min.)
0.3% Nupercain (15 min.)
Tincture of iodine (2 sec.)
Mercurochrome (10 min.)
Trypaflavin (10 min.)
Pyoctanin (5 min.)
Acetone (15 sec.)
Ether (15 sec.)
Chloroform (15 sec.)
Butyl alcohol (15 sec.)
Ethyl alcohol (15 sec.)
Ringer's solution (2.5 hrs.)
0.8 M NaCl (30 min.)
0.5 M KCl (30 min.)
0.5 M CaCl2 (30 min.)
0.5 M MgCl2 (30 min)

Fig. 2. Percentage-wise variations of resistance against the normal values witnessed in the cornea when various chemical stimulations were applied to it.

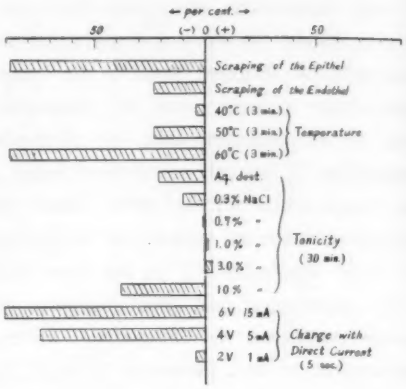

Fig. 3. Changes brought about by physical stimulations.

density was used, the resistance instantaneouly dropped to zero. On the other hand, sulphuric acid of as strong a density as 50% failed to produce as much effect as caustic soda of 2.5% density did. Figure 2 shows a comparative five-second test results between caustic soda and sulphuric acid actions of 1.2% and 12.0% respective densities. The sulphuric acid, though ten times as strong as the caustic soda, is only able to produce half as much effect as the cautic soda.

As we experimented with ophthalmic metal-adstrigents, the resistance showed a conspicuous rise under stimulation of zinc sulphate, and a fall under caustic silver. Protargolum produced a similer symptom to that of caustic silver though to a much more moderate extent. In the case of cupric nitrate, the resistance stayed rather unstable and computation of the average figure was impossible.

Now turning to the topical anaesthetics, the resistance rose to a conspicuosly high point under cocainum hydrochloricum and showed a moderate rise with Cornecain (Procainum hydrochloricum) and Xylocain (Lidocaine hydrochloride) and dropped under Nupercain (percain). The proecss of these experiments against lapse of time shall be reported in a latter paragraph.

Among the antiseptic solutions, tincture of iodine, mercurochrome and pyoctanin (methylviolitt) decreased the resistance, and especially tincture of iodine was found to work by far the strongest action, Trypaflavin (acriflavin), on the other hand, increased the resistance.

Efforts were made to examine the test results reported by Katzin in connection with

the influence worked by acetone and ether and such like upon the cornea resistance. Acetone and ethyl alcohol reduced the resistance as much as 90% below normal within 15 seconds. The decrease of the resistance became less and less conspicuous in the order of chloroform, butyl alcohol and ether ranging from 70% to 40%. From these results we came to know that such decrease of the resistance under the stimulation of various chemicals was closely related with the existance of large amount of fat component in the epithelium, and with the denaturation of protein in the tissue.

Then we conducted another experiment and examined the influence of natrium, kalium, calciun and magnesium inos in the density condition of 0.5M. Calcium increased the resistance as much as 23% and calium brought it down by 50%, which did not correspond with the test results reported by Cogan and Holt.

Figure 3 shows the test results prodced mainly through physical stimulations.

When the epithelium and endothelium were scratched off to the same extent, it was noticed that the destruction of the former brought more marked effect than that of the latter.

Studying the influence of temperatures, it was found that within three-minute test period under temperatures up to 40°C the resistance remained stable and with their rise above 40°C the resistance suddenly dropped.

A 30-minute comparative test of osmotic pressure effect was conducted and it was proved that no change occurred in NaCl of density range from 0.8% to 1.3% and more remarkble change wa sobserved in the solution of 10% density than in distilled water.

In the final stage of our test series, direct current was connected to the epithelium and the endothelium of the extracted cornea and we found the resistance showed a sharp 90% drop at 4 V and 6 V electric current lasting for five seconds. But 2 V current failed to produce any noticeable change. It must be added that the change of the positive and negative electrodes between the epithelium and the endothelium had no specific reflection upon the resistance.

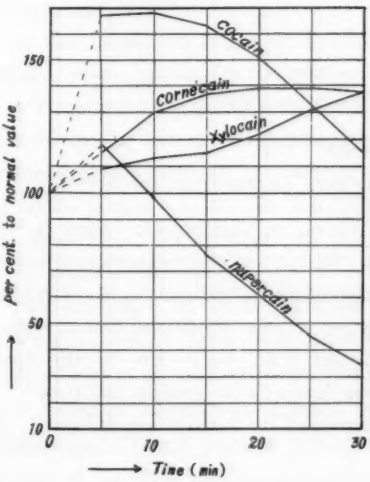

Fig. 4. The average per minute test process when the topical anaesthetic influences were studied.

In the foregoing paragraphs, we have mentioned how we were able to observe the variations of the ion permeability of the corneal tissue itself through different electrical resistances under various conditions. One of the most advantageous aspects of this method is that no more than one test material is required for observation of the entire process to the final stage. Figure 4 shows the average

per minute test process that we reported in a previous parapraph where the topical anaesthetic influences were studied.

Conclusion

We have mentioned the test method of cornea impedance and its practical meaning.

Briefly to review our experiments, we in the first place determined the phase angle of the normal cornea and compared it with the test results of other tissues. And then we brought it under various conditions to see the resulting changes in the cornea phase angle.

Then we stated what influence had been brought upon the resistive property, one of the factors in the corneal impedance, by different physical or chemical stimulations.

On the basis of these findings, we have reached the conclusion that the variations of the cornea permeability can accurately be traced.

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THE ARTHUS PHENOMENON IN THE CORNEA

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When the Arthus phenomenon is experimented on in the cornea which holds no vessels, it should be different from that in the skin. In the model experiments of keratitis parenchymatosa diffusa, Wessely, Arisawa-Szily and other investigators in Japan have reported severe reactions in the cornea from contact of the sensitized tissues with the specific antigen. But, on the other hand, A.R. Rich and R.H. Follis are of the opinion that no reaction can be produced in the intact cornea in which no vessels are found.

Which is right, is a question that must be answered by further experiments. This experiment was designed to solve this question.

Material and Methods

The rabbits were sensitized thoroughly with eggwhite albumin or horse-serum. When

their skins were found severly sensible to the antigen and plenty of antibody were also demonstrated in their serum in vitro by precipitation, 0.1 cc. of 1% eggwhite albumin or full horse-serum was injected into the substance of the cornea. In some experiments trypan-blue or indian-ink, mixed with the antigen, was also injected.

Clinical observations and histological examinations were done at each end of 1/2, 1, 2, 4, 6, 8, 24, 48 hours. Histological sections were stained with Hx-E, toluidin-blue, phospho-wolfrum acid-Hx and by the method of Azan-Mallory.

Results

1) By the clinical observations no differences were found within the first 4 hours between the sensitized and the normal control animals. But later, striking differences were observed as in previous many literatures of the same kind.

2) From the histological observations following results were obtained.

At the area injected with the antigen each collagene fiber of the cornea became severely edematously swollen within the first 1-2 hours, much earlier than in the normal control rabbits, and simultaneously the nuclei of the corneal cells became also swollen and faintly stained. In the control animals the collagene fibers were not so much swollen, but seperated from each other by fluid and so the tissue became loose. Thus the clinical appearances of both corneas showed similar opacities and no pronounced differences.

Then, the keratocytes became gradually stained weaker and weaker, untill they disappeared, and new keratocytes with strongly stained nuclei appeared in their places. In the adjacent parts surrounding this area, such alternations could not or only scarcely be found, except slight looseness.

In the vessels at the limbus, hyaline thrombus appeared simultaneously with the reactions in the cornea (in the section at 1/2 hour) and then plenty of leucocytes migrated out of these vessels into the sub-epithelial tissue and superficial parenchyma of the cornea, distinctly earlier and more abundant than in control animals.

The alterations could be observed at the iris, i. e. the early edematous swelling of the wall of its vessels and the fibrin-meshwork in the anterior chamber, which later condensed to a membrane and covered it. But in the control animals such changes did not appear in their anterior chamber.

The migration of leucocytes from the limbal vessels into the central area was later than the disappearance of the swollen keratocytes. So the cells which had dark nuclei and took the places of the disappeared cells were not the emigrated cells out of the vessels.

Trypanblue which was injected with the antigen was never restricted through the injected region, but it reached promptly to the limbus, the angle of the chamber, the iris and so on. No differences were found in this respect between the sensitized and the normal control animals. The phagocytosis of the keratocyte was observed and no differences were also found between them.

Discussion

The results of this experiment were as follows. (1) When the antigen-antibody reaction occured in the avascular tissue such as cornea, the histological changes were different from that in the usual tissues which contain vessels such as the skin. (2) Its characteristics were the swelling of the connective fibers and the connective cells. In this experiment, became necrotic, faintly stained and later disappeared. (3) The tissue fluid circulation in the cornea was not obstructed by reaction from contact of its tissue with the specific antigen, because the dye injected with the antigen spread as fast as in the control animals. (4) The hyaline thrombus formation in the vessels and earlier migration of the leucocytes out of the vessels were observed in the limbus. (5) The swelling and the necrosis of the cells of the corneal stroma was not due to the disturbances of blood circulations at the limbus and not due to the deprivation of the nutrition from the tissue. There were some less damaged zones between the injected area and the limbus at the early stage of the reaction.

A.R. Rich thought, that the damage of the vessels in the tissue was the real origin of the necrotic reaction of the Arthus phenomenon. He insisted that destruction or obstruction of the vessels deprives the tissue of its nutrition and the tissue cell dies. Because he demonstrated that the necrosis can not be produced in the avascular cornea, but it can be easily observed in the cornea which is previously inflamed and vascularized. His results are different from that of this experiment. It is clear, that whether the necrosis will be produced or not, depends upon the antibody concentration of the tissue, as usually demonstrated in the skin. The intact cornea even of the strongly sensititized rabbits contains only little antibody, but, if once inflamed or invaded by vessels, much antibody should spread out of the vessels into its tissue. Therefore, the vascularized cornea can be damaged to necrosis by antigen-antibody reaction.

The difference between this experiment and his result should be ascribed to the different local antibody quantity.

Opie concluded that in the Arthus phenomenon the tissue cells are directly damaged by contact with the precipitate that forms, when the specific protein meets the precipitin in the tissue fluids. Which is the origin of the damage, precipitate as he said or the act of precipitation, is a question that must be further studied. But from this experiment it can be affirmed that there is in the Arthus phenomenon, beside the vessel damaging factor, another one, which functions directly on the collagene fibers and cells to destroy them through the antigen-antibody reaction.

On the other hand, the observations of Wessely, Arisawa-Szily and others were principally done 24 hours after the injection of the antigen. Therefore the model experiment for keratitis parenchymatosa diffusa has showed mainly the secondary active reactions originated from those in vessels in the limbus and the iris. In this respect, the above mentioned antigen-antibody reactions in the cornea can not be induced to be the direct origin of

this disease.

Summary

1. If the rabbits were sufficiently sensitized, a necrotical change could be observed in the cornea directly due to the antigen-antibody reaction, even though no vessels were previously let invade.

2. This necrotical change was not the indirect reaction due the damage of vessels of the limbus, such as hyaline thrombus were formed and then leucocytes migrated into the corneal stroma.

3. But this necrotical change became covered and indistinguishable with the indirect alterations from limbal vessels within 24 hours after specific antigen was injected.

4. The spread of the antigen in the corneal stroma were not obstructed by the reaction of the antigen-antibody, though it was usually observed in the skin.

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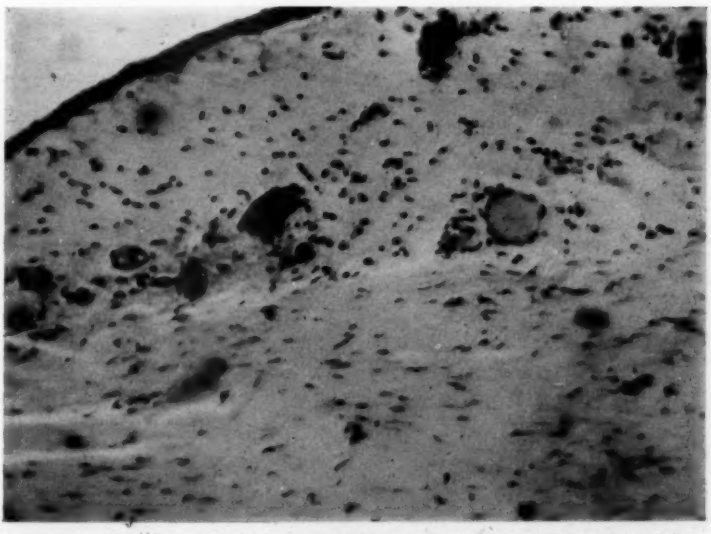

Fig. 1. Subconjunctival tissue near by the limbus of Arthus sensitized animal 2 hours after intracorneal injection of the egg-white albumin. There is hyaline thrombus in the vessel and migration of leucocytes out of the vessel.

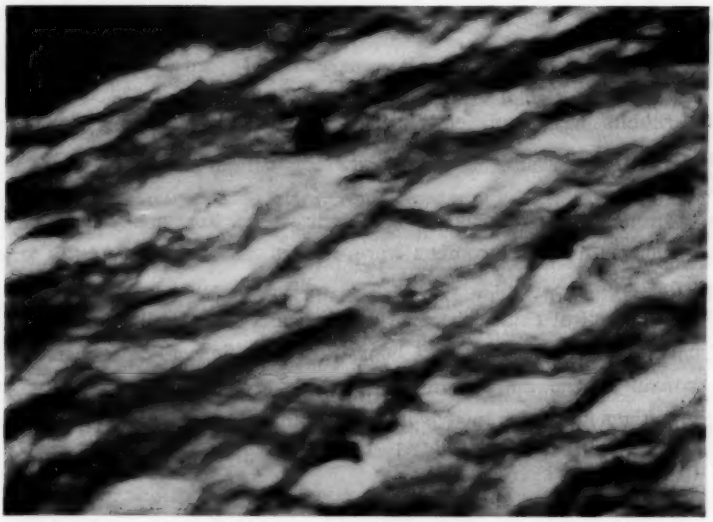

Fig. 2. Corneal stroma of normal control animal 2 hours after intracorneal injection of the antigen. Fibers are separated by fluid, nuclei of the corneal cells appear almost normal and there is no necrosis and no edema of fiber itself.

Fig. 3. Corneal stroma of Arthus sensitized animal 2 hours after the specific antigen injected. There is pronounced edema and swelling of corneal fibers and faintly stained nuclei (X), but no inflammatory cells are present.

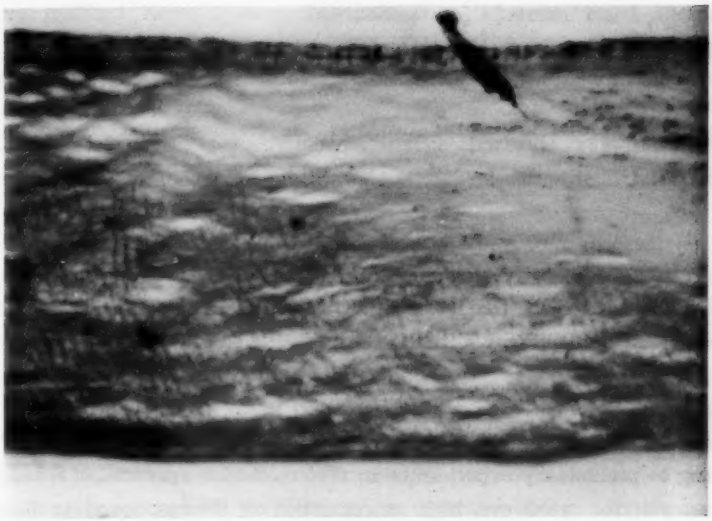

Fig. 4. Peripheral region of the cornea of the eye shown in fig. 3. Leucocytes are migrating into the subepithelial tissue (right side), but there is no noticeable alterations in the corneal substance. It shows that there is an almost intact zone between the injected area and the limbus.

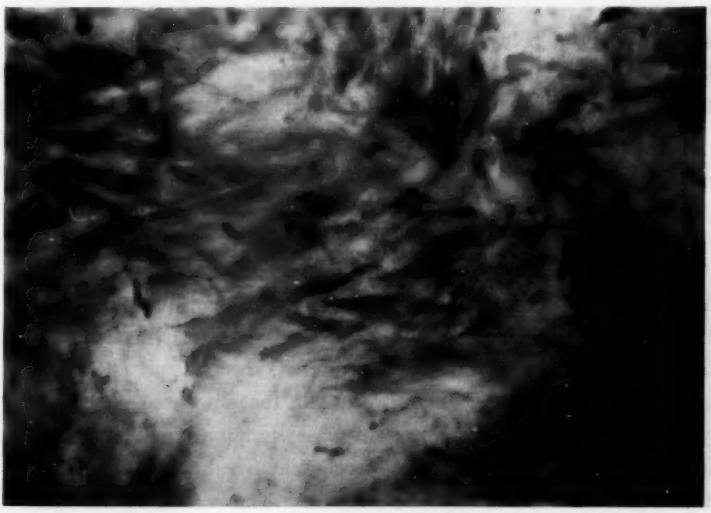

Fig. 5. Cornea near by the injected area of Arthus sensitized rabbit 4 hours after antigen injection. There is edema and sweillng of the corneal fibers. Dark stained cells appear and take place of disapeared cells. They are corneal cells in active condition.

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INCORPORATION OF INORGANIC S^{35}-SULFATE INTO SULFATED MUCOPOLYSACCHARIDES IN THE RABBIT CORNEA

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According to preliminary experiments in vivo on the incorporation of S^{35}-sulfate into the different tissues of the rabbit eye, high concentration of S^{35} was found to be incorporated into sulfated mucopolysaccharides in the corneal stroma. In present investigation in vitro it is the object to determine whether S^{35}-sulfate enters into sulfated mucopolysaccharides of the rabbit cornea, or not.

Method

Excised corneas of white adult rabbits were sliced with a razor blade and used for experiments. Isolations of polysaccharide were conducted as follows.

(1). Extraction method by T. Sato et al. Homogenised slices were extracted with dilute alkalin solution by heating at 50° and the extract was deproteinised by heat coagulation and by additon of ammonium sulfate. Sulfated mucopolysaccharides were precipitated with toluidine blue and the resulting precipitate was sufficiently washed with dilute acid solution and distilled water. It was extracted with formamide and then the formamide-soluble fraction was precipitated with methanol in the presence of sodium chloride. The precipitate was once more treated similary with methanol and sodium chloride. Polysaccharides obtained were used for chromatography analysis.

(2). The slices were extracted with 2 per cent sodium hydroxyde solution at O° for 3 days. The resulting viscid liquid was filtered, neutralysed with acetic acid and dialysed against distilled water. The precipitate that appeared during the dialysis was removed by centrifusion and the supernatant was precipitated with 2 volumes of ethanol in the presence of sodium chloride. The precipitate was dissolved in dilute alkali and undissolved matter was removed by centrifusion. The similar procedure was repeated with the supernatant. The solid thus obtained was dissolved in a small amount of veronal buffer and used for paper elctrophoretic analysis.

Chromatography on filter paper. The chromatographic examinations were carried out

with slightly modified Kerby's ascending method on Whatman No. 1 filter paper. The mixture of n-propanol and 1/15M phosphate buffer (PH 6.4) in the proportion of 3 : 7 was utilized as solvent system and the polysaccharides were developed on the filter paper in a refrigerator at 4° for 24 hours.

Electrophoresis on filter paper. A horizontal type apparatus was used. The electrophoresis of sulfated mucopolysaccharides was conducted in veronal buffer at pH 8,6 on Whatman No. 1 paper at 0.25 mA/cm.

Detection of compounds. Sulfated mucopolysaccharides were detected on paper strips by metachromatic staining with toluidine blue and contaminated proteins also were stained by amide black 10B.

Radioactivity of compounds was detected on the autoradiograms which remained in contact with a non screen X-ray film for 5 to 8 days.

Experiments with slices. A vessel containing 0.13gr. of slices and 1 ml. of Krebs Ringer bicarbonate solution, to which about 40μc. of S^{35}-sodium sulfate was added, was shaken at 37° for 3 hours under gas mixture of oxygen (95 per cent) and carbon dioxyde (5 per cent), then the polysaccharides were isolated as described above. As the control experiments the slices heated at 55° or 100° for 15 minutes were used.

Experiments with supernatant. The mixture of 0.154M. potasium chloride, 0.154M. magnesium choride and 1/10M. phosphate buffer at pH 7.0(78 : 2 : 20)was used for basic medium. The slices were homogenised in the cold for 3 minutes (about 8 per cent homogenete) and the clear supernatants were obtaind by centrifusion at 4000 r.p.m. for 20 minutes and then 15000 r.p.m. for 60 minutes. The mixture of 1.25ml. of the supernatant, about 50μc. of S^{35}-sodium sulfate and 3mg. of ATP (sodium salt) was shaken at 37° for 2 hours under oxygen, and at the end of the incubation a small amount of previously isolated polysaccharide of the cornea was added as carrier. The sulfated mucopolysaccharides were isolated by the method of T. Sato et al as discribed above.

Results and Discussion

Chromatographs of the formamide-soluble fractions are shown in fig. 1. The chromatographic figure is devided into two metachromatic spots when the major value of sample was applied on the filter paper. The small spot is considered to be due to chondroitin sulfate on the basis of its Rf value. The Rf values of major spots were less than that of standard chondroitin sulfate, but it was impossible to identify these major spots since the author had no other authentic standard of corneal polysaccharides. The water extract of the formamide-insoluble residue gave neither metachromatic nor autoradiographic spots.

Electrophoretic patterns of corneal polysaccharides are shown in figs. 3 and 4. The

Magnesium sulfate was substituted by magnesium chloride.

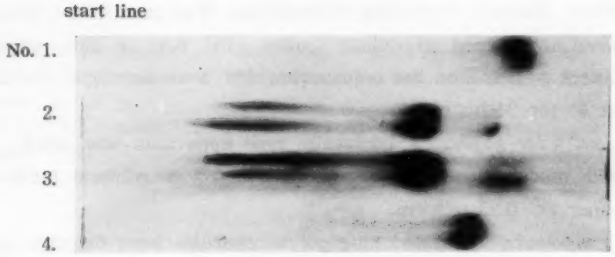

Fig. 1. Paper chromatograms of acid mucopolysacharides in rabbit cornea (No. 2-4) and of chondroitin sulfate (No. 1).
No. 3. Incubation, with slices:
Slices 0.13g.+Krebs-Ringer-bicarbonate (without SO) 1 ml.+S^{35}-sulfate, shakes in the gas mixture of oxygen (95 per cent) and carbon dioxyde (5 per cent) at 37 for 3 hours.
No. 4. Incubation, with supernatant:
Supernatant 1.25 ml.+ATP-Na salt 3 mg. S^{35}-sulfate, shaken in oxygen at 37 for 2 hours, and then added the previously isolated acid mucopolysaccharide of rabbit cornea as carrier.

Fig. 2. Autoradiograms of the paper chromatograms of fig. 1.
No. 1. Incubation with slices.
No. 2. Incubation with supernatant.

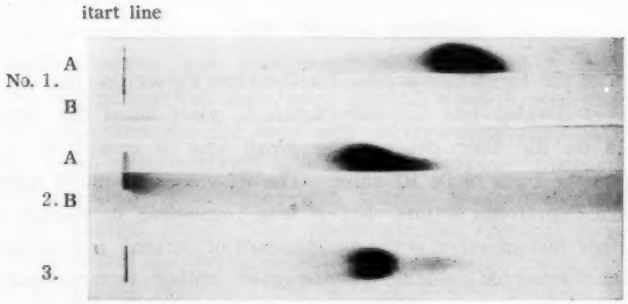

Fig. 3. Electrophoresis on filter paper of acid mucopolysaccharides.
No. 1. Rabbit skeletal cartilage.
No. 2-3. Rabbit cornea.
A: metachromatic staining by toluidine blue.
B: stained by amido black 10 B.

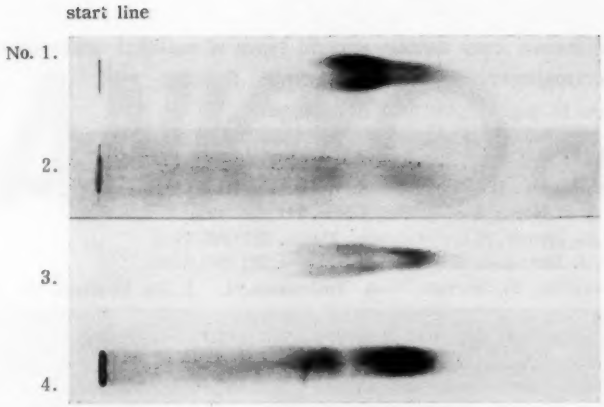

Fig. 4. Comparison of the electrophoretic mobility of acid mucopolysaccharides and of organic S^{35}-sulfate isolated from rabbit cornea.
No. 1-2: Paper stripes.
No. 3-4: Autoradiograms of these paper stripes.
No. 4. remained in contact with paper strip for 10 days.

major portion of corneal polysaccharides migrated electrophoretically slower than rabbit cartilagious chondroitin sulfate and it is considered to correspond to kerato-sulfate from the view point of electrophoretic mobility. Sometimes the small portion appeared as a metachromatic spot with a higher mobility than the main portion of fractions, but the nature of this substance is still obscured. It is concluded from these electrophoretic and chromatographic findings that several types of sulfated mucopolysaccharides are contained in the rabbit cornea, like in the bovin cornea.

Incorporation of inorganic S^{35}-sulfate into corneal acid mucopolysaccharides. The correspondence between metachromatic and their autoradiographic spots are shown in figs 1, 2 and 4. Inorganic S^{35}-sulfate was found to be taken up by the slices of stroma and built into sulfated mucopolysaccharides and similar incorporation also was found to occur when inoganic S^{35}-sulfate was incubated with supernatant of stroma in the presence of ATP sodium salt. The heated slices did not exhibit such an incorporation.

The results of the present investigations lead to the supposition that sulfate activation by ATP is the primary mechanism of sulfate uptake in the cornea as in sulfated compounds in the liver.

Summary

The exchange of sulfate groups of corneal mucopolysaccharides was studied by experiments in vitro. The results were as follows.

1. Radioactive sulfate was taken up by slices and incorporated into sulfated mucopolysaccharides.

2. Similar incorporation also occured by the supernatant of cornea in the presence

of ATP.

3) The rabbit cornea may contain several types of sulfated mucopolysaccharides on the basis of the electrophoretic and chromatographic findings.

(Published in Acta O. Jap. 61:1846, 1957 in Japanese.)

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Correction

In the article "Corneal wave of the rabbit," by Dr. M. Uyemura & K. Kawashima in the January~March issue, 1958, the photographs of Fig. 6 (a), (b) were accidentally interchanged, and also the right was inverted.

Editroial News

In Japan we have two main general meetings for ophthalmologists every year. The one is the Japan Ophthalmological Congress, another the Clinical Ophthalmological Congress.

The Japan Ophthalmological Congress is held in spring at the seat of medical school fixed upon two years before. This year it will give the sixty-second meeting in the city of Niigata during a session of three days (trom May 16 to 18).

Besides ninety ordinary speaches one special and a symposium will be made, the subject of this year's symposium being pathogeny and treatment of squint.

The Clinical Ophthalmological Congress takes place in November every year in Tokyo.

Besides these two main meetings, many large and small sectional are held every year in universities and cities, and the larger ones are as follows:

The North Japan Ophthalmological Congress,
The Tokyo Sectional Meeting (monthly),
The Tohoku Ophthalmological Congress,
The Middle Japan Ophthalmological Congress,
The Chugoku-Shikoku Ophthalmonogical Congress,
The Kyushu Sectional Meeting.

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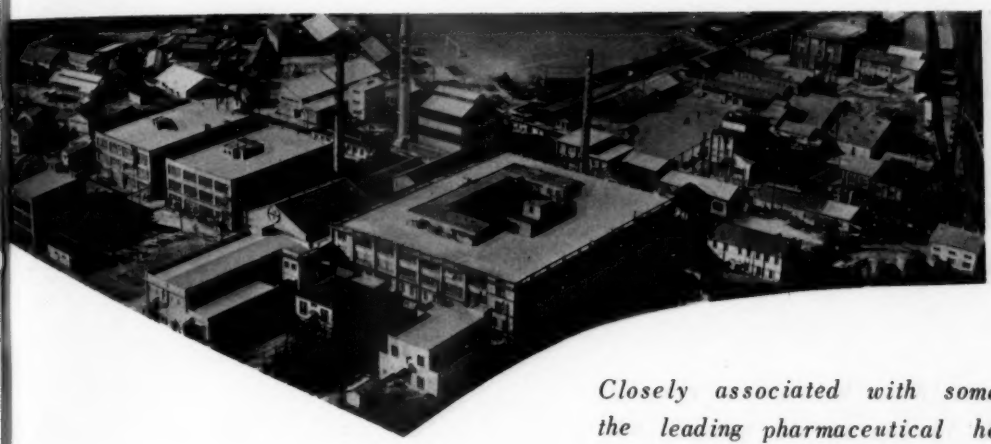

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